



BACILLUS SP. AS POTENTIAL PROBIOTICS FOR USE IN TILAPIA FISH FARMING AQUACULTURE – A REVIEW

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Abstract

Aquaculture is a crucial and rapidly expanding industry in global food production. Fisheries are also an essential socio-economic activity, providing abundant resources and remarkable prospects. However, due to the deteriorating ecological environment, aquatic animals are often exposed to traumatic conditions and are susceptible to bacterial infections that pose significant challenges for aquaculture production. The indiscriminate use of antibiotics in the past has led to the emergence of multidrug-resistant pathogens and sudden outbreaks of infectious diseases, resulting in serious economic losses. Moreover, the use of expensive chemotherapeutic drugs and antibiotics has negative impacts on aquatic environments. Therefore, it is increasingly important to adopt alternative natural agents, such as probiotics and their metabolites, to enhance healthy fish production. Probiotics are microorganisms that have numerous beneficial effects on their hosts. They are environmentally friendly, non-toxic, and cost-effective. This review specifically focuses on the use of *Bacillus* sp. as probiotics to promote healthy tilapia production in the aquatic sector, while also examining their interactions with the immune system and gut microbiota. The information presented in this review can guide future research and promote effective and healthy tilapia culture production.

Key words: *Bacillus* sp., disease resistance, gut health, immune system, tilapia, wastewater treatment

Fish and fishery products are essential sources of protein and micronutrients for human health (Carbone and Faggio, 2016). Tilapia (*Oreochromis niloticus*) is the second most farmed fish globally, it is a freshwater species (Narimbi et al., 2018; Trosvik et al., 2013). The production of tilapia has steadily increased with the growing demand and market value (Andriani et al., 2019; Lum-sangkul et al., 2022; Elumalai et al., 2021; Kurian et al., 2021; Van Doan et al., 2019). Tilapia is a resilient fish that can thrive in various environments, grow rapidly, and withstand challenging conditions (FAO, 2018). The increase in production has also led to a rise in infections and disease outbreaks, resulting in significant economic losses (Van Hai, 2015 a, b; Abarike et al., 2018 a; Joffre et al., 2018; Elabd et al., 2022; Hamed et al., 2021). The most frequently identified infection agent in Nile tilapia culture is *Streptococcus agalactiae* (Van Doan et al., 2018). Various bacteria, including *Streptococcus* spp., *Francisella orientalis*, *Edwardsiella* spp., and *Vibrio* spp., can cause severe skin lesions, septicemia, nervous system damage, and meningoencephalitis, resulting in high economic losses in tilapia farming (Xu et al., 2019).

Intensive feeding practices, high stocking densities, disease outbreaks, and environmental and management issues can lead to increased mortality rates in farmed tilapia (Lee et al., 2019; Dawood et al., 2020). Aquaculture infections can be caused by bacteria, viruses, and parasites (Carbone and Faggio, 2016; Bastos Gomes et al., 2017). Fish commonly contract bacterial infections from *Vibrio* sp., *Streptococcus* sp., *Aeromonas* sp., *Pseudomonas* sp., *Clostridium* sp., *Yersinia* sp., *Acinetobacter* sp., and *Lactococcus* sp. (Santos et al., 2018; Yi et al., 2018). Researchers have recently been exploring ways to strengthen the immune systems of fish as a means of managing and preventing disease outbreaks (Cerezuela et al., 2012). Phagocytosis is a crucial element of the non-specific immune response, as it recognizes and breaks down pathogens and other foreign substances (Secombes, 1990; Steinhagen and Jendrysek, 1994). The use of antibiotics in aquaculture for disease control and growth promotion can alter intestinal microbiota and increase bacterial resistance, which can be harmful or toxic to aquatic species (Dawood et al., 2018; Elumalai et al., 2020; Hoseinifar et al., 2020). To counter this, various

subsequent processes are being used, including probiotics, prebiotics, para-probiotics, vaccines, and medicinal plants (Pérez-Sánchez et al., 2014; Van Hai, 2015 a, b; Abarike et al., 2018 b; Choudhury and Kamilya, 2018; Kuebutornye et al., 2019). Potential probiotic strains possess various characteristic features that enable them to survive the harsh conditions of the gastrointestinal tract. These features include tolerance to acid and bile salts, resistance to gastric juices, ability to produce extracellular enzymes and antimicrobial components that can kill or suppress the growth of pathogens *in vitro*, capability to adhere to intestinal mucus, and ability to colonize the gut (Ringø, 2020). Furthermore, these probiotic strains must meet biosafety requirements, such as non-haemolytic activity and susceptibility to antibiotics, among others (Ringø, 2020). Probiotics offer a promising solution for aquaculture as they bring numerous benefits in comparison to other preventive measures. They provide support in improving fish growth and health, while also protecting the environment. Using probiotics can help boost disease resistance in aquaculture (Abarike et al., 2018 a; Hlordzi et al., 2020; Van Hai, 2015 a, b). Numerous types of bacterial genomes are regulated by bioproducts, which govern bacterial functions, physiology, and cell development. (Grubbs et al., 2017). It is important to conduct a comparative genomic experiment for a specific probiotic strain. Generalizing the results may not be accurate (Lehri et al., 2017). Dietary administration of *B. subtilis* probiotics had a significant impact on the phagocytic and respiratory burst functions of tilapia (Aly et al., 2008 a). There are various types of probiotic *Bacillus* species, each with unique characteristics such as the ability to produce non-pathogenic and nontoxic substances, improve the quality of water, and withstand harsh conditions due to their sporulation capability. These traits differentiate them from other probiotics and provide them with advantages such as heat tolerance and longer shelf-life (Kavitha et al., 2018; Buruiană et al., 2014; Kuebutornye et al., 2019; Geng et al., 2012). Probiotic *Bacillus* species used in aquaculture are sourced from

various places, including soil, water, decaying matter, the gastrointestinal tract of fish and other vertebrates, and commercial sources. Over the years, *Bacillus* sp. has demonstrated its benefits in the aquaculture industry, and several studies have reported its positive effects in Nile tilapia culture (Sumon et al., 2018; Adorian et al., 2018; Sankar et al., 2017; Kuebutornye et al., 2019; Han et al., 2015; Wang et al., 2017) and enhancement of immune function and promotion of growth in other fish species (Irianto and Austin, 2002). In addition, they aid in the development of the host's digestive system, maintain the balance of intestinal microbiota, and influence the structure of the intestines (Allameh et al., 2016; Dagá et al., 2013). Therefore, probiotics have become vital ingredients in feed supplements, while improving the immune system and growth functions as above-mentioned, also inhibiting the growth of pathogens (Amir et al., 2019; Kuebutornye et al., 2020 a; Tachibana et al., 2021; Moustafa et al., 2021; El-Saadony et al., 2021; Liu et al., 2021; Hassaan et al., 2021). They are a successful and environmentally friendly alternative to antibiotics, improving rearing water quality and the fish's response to stress (Elsabagh et al., 2018). Figure 1 illustrates the difference between live probiotic cells and their dead or inactive counterparts, known as paraprobiotics or post-biotics. Paraprobiotics are nonviable probiotic cells with either whole or broken cell structure that provide certain beneficial effects for the host. These beneficial microbes lose their capability due to exposure to various factors that can modify their cell structure, such as severed DNA filaments, a disorder in the cell membrane, or mechanical damage in the cell envelope (Zendeboodi et al., 2020; de Almada et al., 2016). Currently, there is limited understanding of the impact of probiotics on single or mixed *Bacillus* strains when rearing tilapia in the environmental conditions of tilapia farms. As such, this review aims to investigate the effects of utilizing probiotic *Bacillus* strains to enhance the quality of water, intestinal health, disease resistance, immune system, and growth performance of farmed tilapia (El-Saadony et al., 2021).

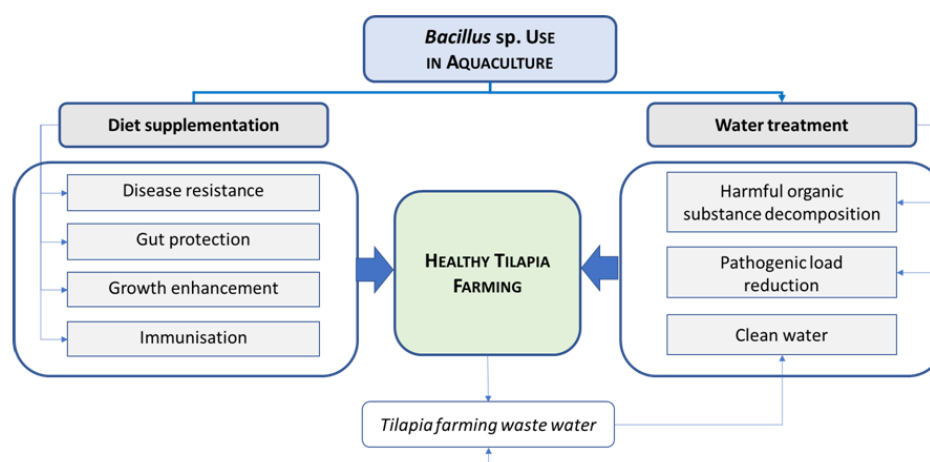


Figure 1. Role of *Bacillus* sp. tilapia fish farming

Table 1. Applications of *Bacillus* species as diet supplements in tilapia fish

<i>Bacillus</i> species	Source	Doses	Fish species	Pathogens	Applications	References
<i>B. subtilis</i>	Commercial	3.9×10 ⁷ CFU/g	Nile tilapia	<i>A. hydrophila</i>	Immuno-stimulation	(Addo et al., 2017)
		1.5×10 ⁹ CFU/g	Nile tilapia	<i>A. hydrophila</i>		(Iwashita et al., 2015)
	NM	NM	Nile tilapia	<i>Flavobacterium columnare</i>	Immuno-stimulation	(Mohamed and Refat, 2011)
		1.1×10 ⁵ CFU/g	Nile tilapia	NM	Immuno-stimulation	(Hassaan et al., 2018)
		10 ⁶ and 10 ¹² CFU/g diet fed	Nile tilapia	<i>S. iniae</i>	Disease resistance	(Aly et al., 2008 b)
		2.05×10 ¹⁰ CFU/kg	Yoshitomi tilapia	<i>A. hydrophila</i>	Disease resistance	(Tang et al., 2017)
<i>B. subtilis</i> SB3615	NM	4×10 ⁷ CFU/g	Nile tilapia	<i>S. iniae</i>	Disease resistance	(Addo et al., 2016)
<i>B. amyloliquefaciens</i>	Commercial	1×10 ⁶ CFU/g	Nile tilapia	<i>Clostridium perfringens</i>	Immuno-stimulation	(Selim and Reda, 2015)
	NM	10 ⁶ –10 ⁷ CFU/g	Nile tilapia	<i>A. hydrophila</i>	Disease resistance	(Chen et al., 2019)
<i>B. cereus</i>	NM	0.5%	Juvenile Nile tilapia	NM	Increase Hb	(Garcia-Marengoni et al., 2015)
<i>B. licheniformis</i> Dahb1 (HM235407.1)	NM	1×10 ⁷ CFU/g	Mozambique tilapia	<i>A. hydrophila</i>	Disease resistance	(Gobi et al., 2018)
<i>B. licheniformis</i>	NM	0.1%	Hybrid red tilapia	<i>S. agalactiae</i>	Disease resistance	(Ng et al., 2014)
<i>B. pumilus</i> AQAH-BS01	NM	1×10 ⁷ –10 ⁹ CFU/g	Nile tilapia	<i>S. agalactiae</i>	Disease resistance	(Srisapoom and Areecho, 2017)
<i>B. subtilis</i> and <i>B. licheniformis</i>	Commercial	5–10 g/kg	Nile tilapia	<i>S. agalactiae</i>	Immuno-stimulation	(Abarike et al., 2018 a, b)
<i>B. cereus</i> and <i>B. subtilis</i>	NM	0.5%	Nile tilapia	NM	Increase HCT	(Garcia-Marengoni et al., 2015)

NM: not mentioned; NA: not applicable, HCT: haematocrit; Hb: haemoglobin, CFU: colony forming unit.

Dietary supplementation

Probiotic *Bacillus* species have become popular in aquaculture due to their ability to withstand unfavourable conditions through sporulation. Feed supplements containing these species are non-pathogenic, environmentally friendly, and have little toxic effects (Zendeboodi et al., 2020). They also may produce antimicrobial components that fight pathogenic infections and secrete hydrolytic enzymes to improve nutrient utilization (Zendeboodi et al., 2020). During a 4–8 weeks trial, Nile tilapia was fed with probiotic *B. subtilis* HAINUP40 (10⁸ CFU/g) as an additive. The probiotic significantly improved growth, immunity, and disease resistance (de Almada et al., 2016). The probiotic *B. subtilis* supplement is used in tilapia culture at different concentrations, such as 3 g/kg⁻¹ (BS3), 5 g/kg⁻¹ (BS5), 7 g/kg⁻¹ (BS7), and 10 g/kg⁻¹ (BS10). When added to 10 g/kg⁻¹ (BS10) diets, it can improve the growth, immune system, and infection resistance against *S. agalactiae* infection in tilapia (Kuebutornye et al., 2019). Dietary inclusion of probiotic *B. licheniformis* Dahb1 at a concentration of 10⁷ CFU/g can enhance the immune system, antioxidant activity, and infection tolerance in Mozambique tilapia against *Aeromonas hydrophila* during a 4-week trial period (Liu

et al., 2017). *Bacillus* sp. NP5 probiotic and paraprobiotic supplements improved Nile tilapia's immune system, survival rate, and infection resistance against *S. agalactiae* in a 30-day trial (Gobi et al., 2018). During an 84-day trial period, Nile tilapia was fed a diet that included a probiotic supplement consisting of 1.85 × 10⁵ *B. pumilus* CFU/kg of diet and 0.5 g of protease kg⁻¹. After the trial period, it was observed that the immune response, intestinal histological morphometric, growth performance, haematological, serum biochemical, metabolic gene expression, and intestinal bacterial flora of the tilapia were significantly improved. This positive outcome was achieved despite the diet being free of fish meal (Mulyadin et al., 2021). Dietary inclusion of *Bacillus* probiotics (10⁸ CFU/g) enhanced growth, feed utilization, and disease resistance against *A. hydrophila* in Nile tilapia over a 30-day trial (Hassaan et al., 2021). During a feeding experiment in tilapia farming, the effects of different concentrations of probiotic *B. subtilis* LT3-1 were studied. The concentrations used were 3.8 × 10¹⁰ (B1), 7.6 × 10¹⁰ (B2), 1.14 × 10¹¹ (B3), and 1.52 × 10¹¹ (B4) CFU/kg, compared to a reference feeding without probiotics, over six weeks. The results showed that the optimal concentration of probiotic *B. subtilis* LT3-1

(3.8×10^{10} – 7.6×10^{10} CFU/kg) significantly improved growth and the immune system of tilapia. Additionally, it provided increased resistance against *S. agalactiae* infection in tilapia farming (Samson et al., 2020). There is limited information available on the efficiency of probiotic *Bacillus* species in Nile tilapia farming in terms of their effects on gut health and mucosal immunity, particularly their host connection (Kuebutornye et al., 2019). Dietary inclusion of probiotics *B. subtilis* TPS4, *B. velezensis* TPS3N, and *B. amyloliquefaciens* TPS17, either alone or in combination, significantly improved tilapia farming by enhancing mucosal immunity, intestinal health, and disease tolerance towards *A. hydrophila* infection (Zhu et al., 2019). A feeding assessment was conducted to examine different concentrations (0.5% to 2%) of probiotics (Biogen) during 120 days of Nile tilapia farming. The study results showed a significant improvement in growth rate and nutrient efficiency (Kuebutornye et al., 2020 b, c). Table 1 lists some applications of single and combined *Bacillus* species and strains as diet supplements for tilapia fish.

Mechanisms and impact on tilapia health

Gastrointestinal tract of tilapia

The intestinal microbiome has a substantial impact on the maintenance of gut well-being and the metabolism of the individuals it inhabits. The intestinal lining is influenced by various internal elements, such as feeding patterns, as well as external factors, including the microbial population in water, dietary choices, and the surrounding environment (Nayak 2010 a; Yukgehnash et al., 2020). The intricate and dynamic microbial community within the intestinal tract can be influenced by and has the potential to govern aspects of fish nutrition, well-being, mucosal growth, maturation, metabolic processes, and the ability to resist infections in tilapia (Standen et al., 2015). Numerous investigations have demonstrated the gut microbiota's capacity to establish connections with the host's tissues and oversee functions related to energy metabolism, body weight, fat distribution, insulin sensitivity, and lipid metabolism (Zhang and Zhang, 2013; Falcinelli et al., 2015; Kim et al., 2018). The study by Sánchez et al. (2017) demonstrates the significant contribution of gut bacterial populations. These contributions include fortifying the epithelial barrier, restraining the proliferation of pathogens, promoting the development of gut-associated lymphoid tissue, breaking down indigestible polysaccharides, and producing essential bio-products such as vitamins, fatty acids, amino acids, and bacteriocins (Sánchez et al., 2017). The gut microbiota has been recognized as a biological indicator for assessing nutritional well-being, nutrient absorption, and the reinforcement of the immune system in fish (Adeoye et al., 2016; Ray et al., 2012; Niu et al., 2019). The influence of the intestinal microbial community is pivotal in the context of dietary supplements for fish (Zhang et al., 2019 a). Probiotics for fish have a beneficial role in enhancing gut microbial equilibrium and modifying

the innate immune responses in fish (Liu et al., 2016). Beneficial gut microbes serve a crucial role in preserving the host's intestinal microbial equilibrium by replacing harmful bacteria with beneficial ones and thwarting the colonization of pathogens in the gut. The specific choice of beneficial microbes used determines their effectiveness in achieving this balance (Nayak, 2010 b). The dietary introduction of two probiotics can withstand the digestive process throughout the fish's growth period and can yield various positive impacts on the host (Ringø et al., 2016; Li et al., 2018). The inclusion of probiotic *B. subtilis* in the diet resulted in substantial enhancements in intestinal structure, as well as increased growth and the accumulation of protein and lipids throughout the entire body of tilapia. This outcome was compared with the control group in the study report (Sayes et al., 2018). In a 56-day experiment with Nile tilapia, the addition of probiotic *B. subtilis* C-3102 at a concentration of 2.5×10^5 CFU/g resulted in a remarkable improvement in the composition of the intestinal microbiota. Furthermore, it triggered an increase in the expression of cytokines, specifically IL-1b, TGF- β , and TNF- α , and a decrease in the expression of intestinal HSP70, as reported in a study (Adeoye et al., 2016 a, b).

Disease resistance

Probiotic *Bacillus* species demonstrate a strong inhibitory activity against a wide range of fish pathogens, including *Aeromonas*, *Vibrio*, *Streptococcus*, *Yersinia*, *Pseudomonas*, *Clostridium*, *Acinetobacter*, *Edwardsiella*, *Flavobacterium*, white spot syndrome virus, and communicable hypodermal and hematopoietic necrosis virus. These inhibitory effects are achieved through various mechanisms, including the synthesis of bacteriocins, regulation of virulence gene expression, competition for adhesion sites, production of lytic enzymes, synthesis of antibiotics, immunostimulation, nutrient and energy competition, and the production of organic acids. These multifaceted mechanisms contribute to the enhanced ability to combat pathogens in the aquaculture industry (He et al., 2013). Kuebutornye et al. (2020 d) investigated the identification and characterization of probiotics derived from the Nile tilapia fish gut, specifically *B. subtilis* TPS4 (MK130899), *B. velezensis* TPS3N (MK130897), and *B. amyloliquefaciens* TPS17 (MK130898). These probiotics displayed susceptibility to a variety of antibiotics, including gentamicin, penicillin, ampicillin, tetracycline, cephalixin, chloramphenicol, kanamycin, ceftriaxone, amikacin, cefoperazone, erythromycin, doxycycline, ciprofloxacin, and clindamycin (except TPS4), and furazolidone (except TPS17) (Kuebutornye et al., 2020 c). Moreover, these probiotics exhibited strong antimicrobial effects against various fish pathogens, including *S. agalactiae*, *A. hydrophila*, and *Vibrio harveyi*. The results of the study suggest that probiotic *Bacillus* species have significant potential for application in tilapia aquaculture, as they can contribute to enhancing disease resistance and overall health in these fish (Kuebutornye

et al., 2020 b). The addition of microencapsulated probiotic *Bacillus* sp. NP5 to the diet significantly enhanced immune responses, promoted growth, and increased resistance to *S. agalactiae* infection in tilapia culture (Agung and Yuhana, 2015). Probiotic *Bacillus* species exhibit strong antagonistic properties against pathogens, are capable of synthesizing beneficial metabolites and antibiotics, possess immunomodulatory abilities, and can effectively contribute to disease control in aquaculture (Al-Ajlani and Hasnain, 2010; Amin et al., 2015; McKee et al., 1985; Silo-Suh et al., 1994). The dietary inclusion of the potential probiotic *B. velezensis* LF01 strain demonstrated the most robust antagonistic effect against *S. agalactiae*. Additionally, the probiotic's bioproduct exhibited substantial antimicrobial activity against a variety of fish pathogens, including *S. agalactiae*, *A. hydrophila*, *Streptococcus iniae*, *Edwardsiella tarda*, *Edwardsiella ictaluri*, *A. schubertii*, *A. veronii*, *A. jandaei*, and *Vibrio harveyi* in tilapia culture. As a result, the inclusion of the probiotic *Bacillus velezensis* LF01 in the diet significantly enhanced the infection resistance to *S. agalactiae* in tilapia culture (Zhang et al., 2019 b). The inclusion of a potential probiotic *Bacillus* species combination in the diet exhibits superior immunomodulatory effects in countering *S. iniae* infection within the context of tilapia culture (Moustafa et al., 2021). The inclusion of the probiotic *B. subtilis* strain HAINUP40 in the diet has been found to enhance disease resistance against *S. agalactiae* in tilapia culture (Zhao, 2014).

Immune system enhancement of tilapia

The addition of single or combined probiotic and exogenous enzyme diet supplements has demonstrated a significant improvement in the immune system, including increased phagocytic and lysozyme activity in tilapia (Hassaan et al., 2021). These enhancements in phagocytic and lysozyme functions have significantly fortified the immune system of fish, making them more effective in defending against harmful microbes (MacArthur and Fletcher, 1985). The incorporation of a diet containing probiotic *Bacillus* species has been shown to enhance the immune system of fish (Tort et al., 2003; Magnadóttir, 2006; Uribe et al., 2011; Thompson, 2017; Wilson, 2017). Probiotic *B. subtilis* C-3102 has been found to produce bioproducts such as β -glucan and bacteriocins, which are included in diet supplements to promote the stimulation of the immune system in tilapia (He et al., 2013). The addition of *B. subtilis* NZ86 or O14VRQ to the diet has significantly enhanced the immune system's response against pathogens in tilapia culture (Galagarza et al., 2018). In a 50-day experiment, the dietary incorporation of marine thermotolerant probiotic *B. paralicheniformis* SO-1 at a concentration of 5–10 g/kg resulted in notable improvements in the immune system's capacity, feed digestion, and overall performance in tilapia (Makled et al., 2019). Dietary inclusion of probiotic *Bacillus* species has been shown to enhance the immune system, with a particular increase in globulin levels in Nile tilapia

(Elsabagh et al., 2018; Zhou et al., 2010). In a feeding trial lasting 84 days, the dietary administration of *Bacillus subtilis* at a concentration of 5×10^6 CFU/g of feed notably improved the nonspecific immune system, including lysozyme and phagocytic activities of macrophages. Additionally, it helped reduce stress associated with high stocking densities in Nile tilapia (Telli et al., 2014). The dietary inclusion of *B. subtilis* strains NZ86 or O14VRQ at 51 days in a feeding trial significantly enhanced the innate immune response, phagocytic, respiratory, and complement activities, as well as upregulated the expression of proinflammatory and cytokine genes in Nile tilapia (Galagarza et al., 2018). In a separate study, the oral dietary administration of *B. subtilis* spores exposed to specific immunogenic proteins improved the immune system and increased resistance against *S. agalactiae* in Nile tilapia (Yao et al., 2019). Furthermore, the probiotic *B. amyloliquefaciens*, when added at concentrations of 10^6 and 10^4 CFU/g after a 30-day feeding trial, particularly increased serum lysozyme activity, enhanced serum killing, elevated serum nitric oxide levels, and raised interleukin-1 (IL-1) and tumor necrosis factor α (TNF α) mRNA levels in anterior kidney tissues. This led to improved disease resistance against *Clostridium perfringens* type D or *Yersinia ruckeri* in Nile tilapia (Selim and Reda, 2015). In yet another study, the dietary incorporation of *B. cereus* at 1.0×10^7 CFU/g during a 42-day trial period significantly improved the immune system and regulated gut microbiota in Nile tilapia. The direct method of administration was found to be more efficient compared to the indirect method (Wang et al., 2017).

Effect of growth enhancement of tilapia

Dietary incorporation of probiotic *Bacillus amyloliquefaciens* at concentrations of 1×10^4 and 1×10^6 CFU/g during a 60-day trial significantly improved various growth parameters, including body weight (BW), weight gain (WG), specific growth rate (SGR), and food conversion ratio (FCR). Additionally, it led to increased intestinal villi height and helped maintain gut microbiota (Reda and Selim, 2015). In another study, the inclusion of potential probiotic *Bacillus safeness* NPUST1 at concentrations of 10^6 CFU/g and 10^7 CFU/g during an 8-week feeding trial notably improved weight gain, feed efficiency, specific growth rate, and the overall health status of tilapia (110). Furthermore, the dietary administration of potential probiotic *B. licheniformis* at 10^9 CFU/g has been shown to enhance weight gain, feed efficiency, specific growth rate, and overall immunity in tilapia culture. This probiotic presents an alternative approach for both feeding and therapeutic purposes in the aquaculture sector (Wu et al., 2021). The effects of various probiotic *Bacillus* strains in tilapia fish are diverse: probiotic *Bacillus* sp. KUAQ1 and *Bacillus* sp. KUAQ2 dietary supplements did not have a significant impact on standard daily growth, normal weight, exact growth ratio, or feed conversion ratio in tilapia fry following an 8-week feeding trial (Yaquub et al., 2021).

Dietary administration of *B. subtilis* at a concentration of 1.6×10^{10} CFU/g significantly improved weight gain and increased thrombocyte counts in the tilapia bloodstream. This dietary management also led to modifications in the gut microbiota and reduced the presence of potential pathogenic species (Sookchaiyaporn et al., 2020). The dietary inclusion of *B. licheniformis* Dabhl at concentrations of 10^5 – 10^7 CFU/g significantly improved final weight (FW), specific growth ratio (SGR), and feed conversion ratio (FCR) in tilapia fish after a 4-week trial period (Tachibana et al., 2021). Probiotic *B. subtilis* strain HAINUP40 at a concentration of 10^8 CFU/g notably increased the percent weight gain (PWG), final body weight, specific growth ratio (SGR), and significantly reduced feed conversion ratio in Nile tilapia during an 8-week feeding trial (Gobi et al., 2018). Probiotic *B. subtilis* E221 at a concentration of 10^8 CFU/g, administered over an 8-week trial period in Nile tilapia, significantly improved weight gain, specific growth rate, and feed conversion ratio. It also played a role in modulating gut microbiota composition under hypersaline stress (Liu et al., 2017). Dietary supplementation with *B. subtilis* at 4 g/kg during a 12-week feeding trial notably improved average body weight (BW), body length (BL), weight gain (WG), and specific growth ratio (SGR) for Nile tilapia fingerlings under the given experimental conditions (Tang et al., 2020).

Indirect use through water quality management

Probiotic microflora treatments in aquaculture water provide a range of benefits, including:

Reduction of Pathogenic Load: These treatments help decrease the presence of harmful microorganisms in the aquatic environment (Soltan et al., 2016). **Improved Water Quality:** Probiotic *Bacillus* species have been used to significantly enhance water quality in shrimp aquaculture ponds. This enhancement is achieved by reducing levels of ammonia, phosphate, nitrate, nitrite, and chemical oxygen demand, resulting in improved conditions for shrimp growth and harvesting (Xiang-Hong et al., 2003). **Organic Pollutant Reduction:** Probiotic *Bacillus* species are directly applied to pond water to reduce organic pollutants and other contaminants. They work by breaking down organic matter into smaller units, thereby improving water quality. This effect is also beneficial for larval survival and growth in hatcheries (Porubcan, 1991 a, b). **Water Quality Enhancement:** Commercial probiotics such as *B. licheniformis* and *B. subtilis* have notably improved water quality guidelines, meeting acceptable ranges for pH, dissolved oxygen (DO) concentration, and ammonia levels, which are essential for successful fish cultivation (Das et al., 2017). **Nutrient Reduction:** The addition of probiotic *Bacillus* species in aquaculture ponds, with concentrations of 10^9 CFU/g feed/m³, significantly reduces the levels of phosphorus, ammonia, nitrogenous compounds, biochemical oxygen demand (BOD), and chemical oxygen demand (COD). This treat-

ment allows for the reuse of aquaculture wastewater following a bioremediation process, making it suitable for fish and prawn rearing (Loh, 2017). **Alteration of Water Quality Criteria:** Treatment with *B. megaterium* leads to significant changes in water quality criteria such as pH, alkalinity, total dissolved solids (TDS), DO, ammonia, phosphorus, BOD, and COD. Temperature and transparency remain unaffected by the treatment (El-Haroun et al., 2006). **Water Quality Modulation:** *Bacillus* species-treated water can modulate various water quality specifications, including pH, transparency, alkalinity, hardness, TDS, DO, COD, BOD, phosphates, nitrogenous species, heavy metals, oil spillage, and the reduction of pathogenic microorganisms. The bioremediation process involving *Bacillus* species achieves maximum results and is a suitable method for managing wastewater in aquaculture (Hlordzi et al., 2020). **Nitrification:** The addition of *Bacillus* probiotics promotes nitrification, a process that reduces hydrogen ion concentration and contributes to maintaining appropriate pH levels (Camargo and Alonso, 2006). **Organic Matter Removal:** *Bacillus* species are effective in removing organic matter from culture systems, which can help maintain water quality (Luis-Villaseñor et al., 2011). **Involvement in the Nitrogen Cycle:** *Bacillus* species play various roles in the nitrogen cycle, including ammonification, nitrification, denitrification, and nitrogen fixation (Hui et al., 2019; Rout et al., 2017; Verbaendert et al., 2011). For example, *Bacillus amyloliquefaciens* DT transforms organic nitrogen into ammonium, while *B. cereus* PB8 reduces NO₂-N from wastewater (Yousuf et al., 2017; Hui et al., 2019; Barman et al., 2018). **Sludge Reduction:** Probiotic *Bacillus* species in aquaculture wastewater help convert different forms of nitrogen, remove organic pollutants, recycle nutrients in the water column, and reduce sludge accumulation (Soltani et al., 2019). **Metal and Hydrocarbon Degradation:** Certain *Bacillus* strains, such as *B. fusiformis*, can improve low concentrations of Ca²⁺, Mg²⁺, and Fe²⁺ and degrade petroleum hydrocarbons (Dongfeng et al., 2011). In summary, probiotic *Bacillus* species have a significant impact on aquaculture water by enhancing water quality, reducing pollutants, and promoting a more sustainable and healthier aquatic environment. Additional applications of *Bacillus* species to better water quality criteria in aquaculture are listed in Table 2.

Conclusion

Tilapia fish holds significant economic importance in the aquaculture sector, but the growth in its production has been accompanied by increased susceptibility to infections and disease outbreaks, resulting in substantial economic losses. The conventional use of chemotherapeutic agents and antibiotics for disease control has raised concerns about their negative impact on aquatic species and the overall aquatic environment. As an alternative approach, probiotics, particularly those from the *Bacillus* genera, have emerged as promising solutions to promote healthy aquatic production in the field of aquaculture.

Table 2. *Bacillus* species treated to improve water quality parameter in aquaculture

<i>Bacillus</i> species	Sources	Doses	Water quality parameter	Role of applications	References
<i>B. velezensis</i> AP193	Soil and catfish intestine	NS	Total phosphorus, total nitrogen, nitrate	Water	(Thurlow et al., 2019)
<i>Bacillus</i> sp. YB1701	Coastal sediment	1×10 ⁵ CFU/mL	<i>A. hydrophila</i> and <i>Vibrio parahaemolyticus</i> , COD	Water	(Zhou et al., 2018)
<i>B. subtilis</i> FY99-01	Commercial	5×10 ⁴ CFU/mL	pH, nitrite, phosphorus	Water	(Wu et al., 2016)
<i>B. licheniformis</i> , <i>B. subtilis</i> , <i>B. polymyxa</i> , <i>B. laterosporus</i> and <i>B. circulans</i>	Commercial	10 ⁸ CFU/L	Ammonia nitrogen, total bacteria count	Water	(Yaqub et al., 2021)
<i>B. cereus</i> PB88	Shrimp ponds	NS	<i>Vibrio harveyi</i> , <i>Vibrio vulnificus</i> , NO ² -N	Denitrification medium	(Naderi Samani et al., 2016)
<i>B. megaterium</i>	Soil	NS	Copper, iron, zinc, manganese	Heavy metal solution	(Barman et al., 2018)
<i>B. subtilis</i>	Fermented pickles	10 ⁵ and 10 ⁸ CFU/mL	Ammonia, nitrite and nitrate ions	Synthetic pond water, water	(Stefanescu, 2015)

NM: not mentioned; NS: not specified; cfu: colony forming unit; COD: chemical oxygen demand; BOD: biochemical oxygen demand; TDS: total dissolved solids; TAN: total ammonia nitrogen.

Through this review, it has become evident that *Bacillus* probiotics offer a range of advantages and effects in tilapia farming:

Enhanced Disease Resistance: Probiotics have been shown to improve the disease resistance of tilapia, reducing the incidence of infections and disease outbreaks.

Boosted Immune System: Probiotic supplementation enhances the immune system of tilapia, making them more resilient to various pathogens.

Improved Growth Performance: Tilapia treated with probiotics exhibit enhanced growth performance, resulting in larger and healthier fish.

Enhanced Gut Health: Probiotics play a pivotal role in maintaining gut health in tilapia, contributing to better digestion and nutrient absorption.

Enhanced Water Quality: Probiotics, particularly those of the *Bacillus* genus, have been effective in improving water quality in aquaculture settings. They reduce the presence of pollutants, break down organic matter, and mitigate the negative effects of pathogenic microorganisms, resulting in a healthier aquatic environment.

Looking ahead, future innovations in aquaculture may involve various beneficial components, including prebiotics, synbiotics, nutrients, biomolecules, combinations of nanoparticles and probiotics, herbal fermented and mixed probiotics, and biopolymer-encapsulated probiotics. These novel combinations and approaches can further enhance feed additives, leading to healthier and more sustainable tilapia production in the aquaculture sector. By exploring and implementing these innovative strategies, aquaculture can continue to thrive while minimizing the environmental impact and ensuring the health of aquatic species.

Authors contribution

Srirengaraj Vijayaram: conceptualization, writing – original draft, literature search, table preparation, vali-

ation. Chi-Chung Chou: writing – review and editing, supervision, validation. Hary Razafindralambo: writing – review and editing, manuscript outline. Hamed Ghafarifarzanid: writing – review and editing, supervision, validation. Elahe Divsalar: writing – review and editing. Hien Van Doan: writing – review and editing.

Conflict of interest

The authors have no conflict of interest to declare for the publication of the present work.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Data availability statement

The data that support the findings of this study are openly available in ORBI at <https://orbi.uliege.be/my-orbi>. Existing literature was used, as well as open and restricted access papers, peer review journals, relevant reports that are available in literature databases such as ORBI, Google Scholar, and Pubmed.

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