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Review

Unveiling the Impact of the Enigmatic Probacterium (*Bacillus*) on Aquatic Life: A Comprehensive Exploration into Its Effects on Fish and Shellfish

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Abstract: Probiotics are becoming more and more popular in the growing of fin and shellfish because these species are consumed worldwide and because different techniques are employed to maximize the productivity and efficiency of fin and shellfish. Probiotic *Bacillus* is one of the many types of probiotics that are used, but it stands out due to its superior qualities. For instance, because of its ability to sporulate, it can survive in harsh environments, produce antimicrobial substances, and is safe to feed to fish. The different species of *Bacillus* used in the farming of fin and shell fish were discussed in this review, along with the benefits of using *Bacillus spp*. as a good substitute in sustainable aquaculture to improve feed utilization, immune system response, stress adaptation, resistance to infectious diseases, tissue preservation integrity, and water quality. Additionally, we discussed the possible risks to safety that *Bacillus* poses when raising these significant species. For the purpose of future research and development regarding *Bacillus* application in aquaculture, a summary of the findings of recent studies about the advantages of applying *Bacillus* to enhance fin and shellfish aquatic animal culture has been provided.

Keywords: *Bacillus*; finfishes; shellfishes; antibiotics; aquaculture; bacteria; probiotics

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1. Introduction

A significant obstacle to the cultivation and rearing of fin and shellfish is the rise in illnesses among aquaculture species brought on by high stocking levels to meet the enormous demand for fish [1]. Due to the negative effects of antibiotics, such as bacterial resistance to common antimicrobials resulting from changes in the microbiota of aquaculture systems, which in turn affects the naturally occurring beneficial bacteria flora, probiotics have become a viable alternative to antibiotics [2].

The aquaculture of fin and shellfishes has expanded quickly to become a significant global sector that employs hundreds of thousands of trained and unskilled individuals in addition to generating economic revenue and high-quality food products [3]. According to projections, there will be nine billion people on the earth by the year 2050, and aquaculture will play a significant part in meeting the growing demand for food [4]. Finding long-lasting, ecologically acceptable treatments to fin and shellfish disease is a top priority for researchers [5]. As a result of this, over time, a variety of probiotics have been found and used to improve the growth and immunity of aquaculture species. These probiotics include *Arthrobacter*, *Enterococcus*, *Bacillus*, *Lactobacillus*, *Micrococcus*, *Lactococcus*, *Aeromonas*, *Pediococcus*, *Enterobacter*, *Burkholderia*, *Vibrio*, *Rhodopseudomonas*, *Pseudomonas*, *Shewanella* and *Roseobacter* [6]. Probiotics are used as a safe supplement in aquaculture to enhance the host's health through growth promotion, nutrient provision, microbial colonization modulation, immune response enhancement, feed utilization improvement, increased digestibility and activity of digestive enzymes, improved water quality, and disease control [7].

Bacillus species have a twofold advantage in terms of survival (heat tolerance and longer shelf life) in a variety of environments: their capacity for sporulation, which prolongs their period of

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effectiveness, and their ability to produce antimicrobial substances that are effective against a wide range of microbes and are non-pathogenic and non-toxic [8]. *Bacillus* species have been shown to boost the activity of antioxidant and digestive enzymes, as well as the expression of genes linked to stress and immunity, enhancing fish's resistance to pathogenic bacteria [9]. Additionally, *Bacillus* species improve fish feed utilization, which increases growth rate [9,10]. The goal of this review is to gather data on the role of *Bacillus* species in regulating digestive enzymes, antioxidant enzymes, immune, stress, and other related gene expression, hepatic indices, disease resistance, feed utilization and growth, and future prospects of *Bacillus* species in fin fish and shellfish aquaculture, as Figure 1 illustrates.

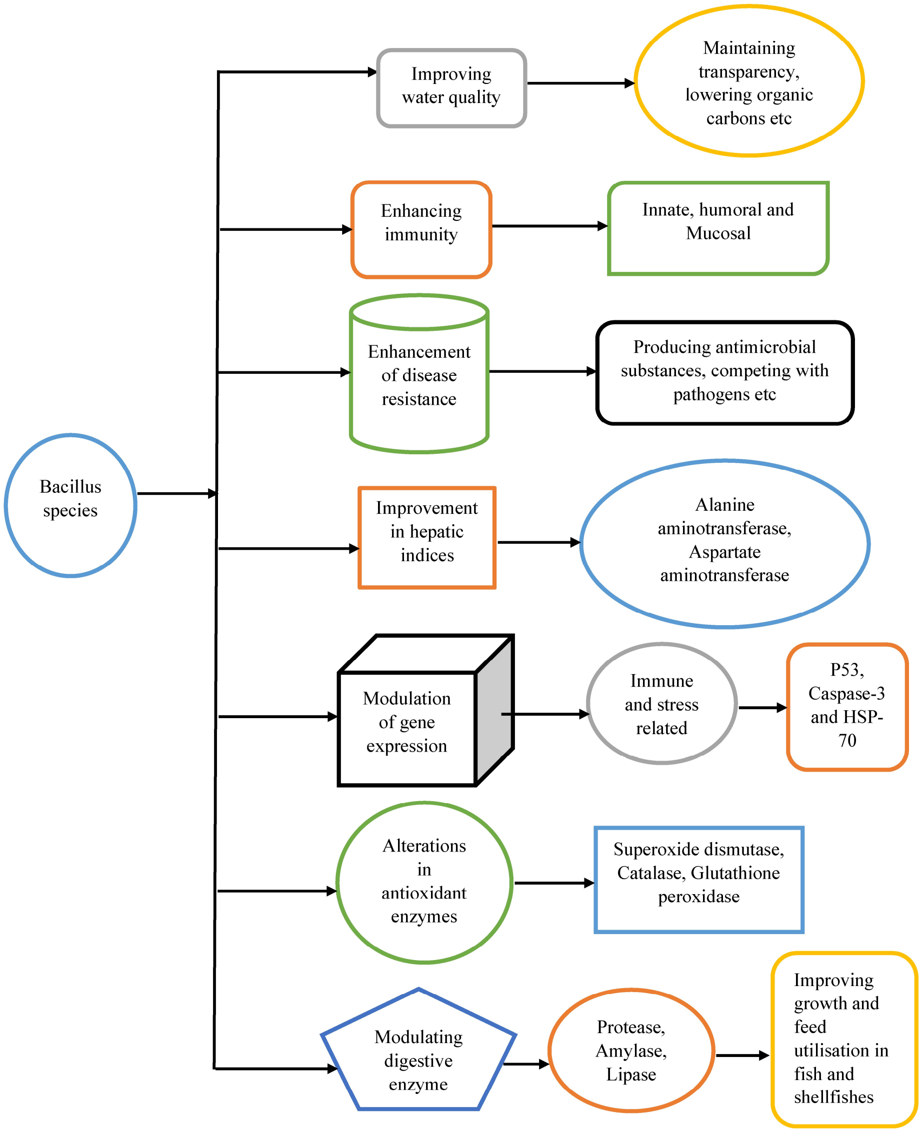

Figure 1. Probiotic *Bacillus* effects on the environment and fin and shell fish Adopted from [7].

2. Characteristics of *Bacillus spp*

One of the most prevalent groups of microorganisms in nature is the *Bacillus* bacteria, they are found in soil, water, and the atmosphere [11]. Figure 2 shows the characteristics of *Bacillus* species that make them viable probiotic options for sustainable growth of fin and shellfish. The ability of *Bacillus*, a diverse group of rod-shaped, Gram-positive bacteria, to produce a potent spore sets them apart. *Bacillus* species are rod-shaped, gram-positive, chemoheterotrophic, aerobic, or facultatively anaerobic, catalase-positive bacteria that are usually motile by peritrichous flagella and do not have

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capsules [12]. *Bacillus* produce spores that can be kidney-shaped, round, oval, or cylindrical and are more resistant to heat, drying, and disinfectants than their vegetative cells [3] thus continue to exist for a long time. Each cell contains one spore, and exposure to air has no effect on sporulation. *Bacillus* species are often beta-hemolytic and grow in large, flat colonies on non-selective media. *Clistridia* and *sporolactobacillus* are not catalase-negative and aerobic, which distinguishes the few genius *Bacillus* species from each other [9]. The capacity of numerous *Bacillus* species to produce antibiotics and other compounds with antagonistic effects on harmful germs makes them significant [4]. Due to their capacity to create antibiotics and other compounds with antagonistic effects on harmful microbes, many *Bacillus* species are significant [16].

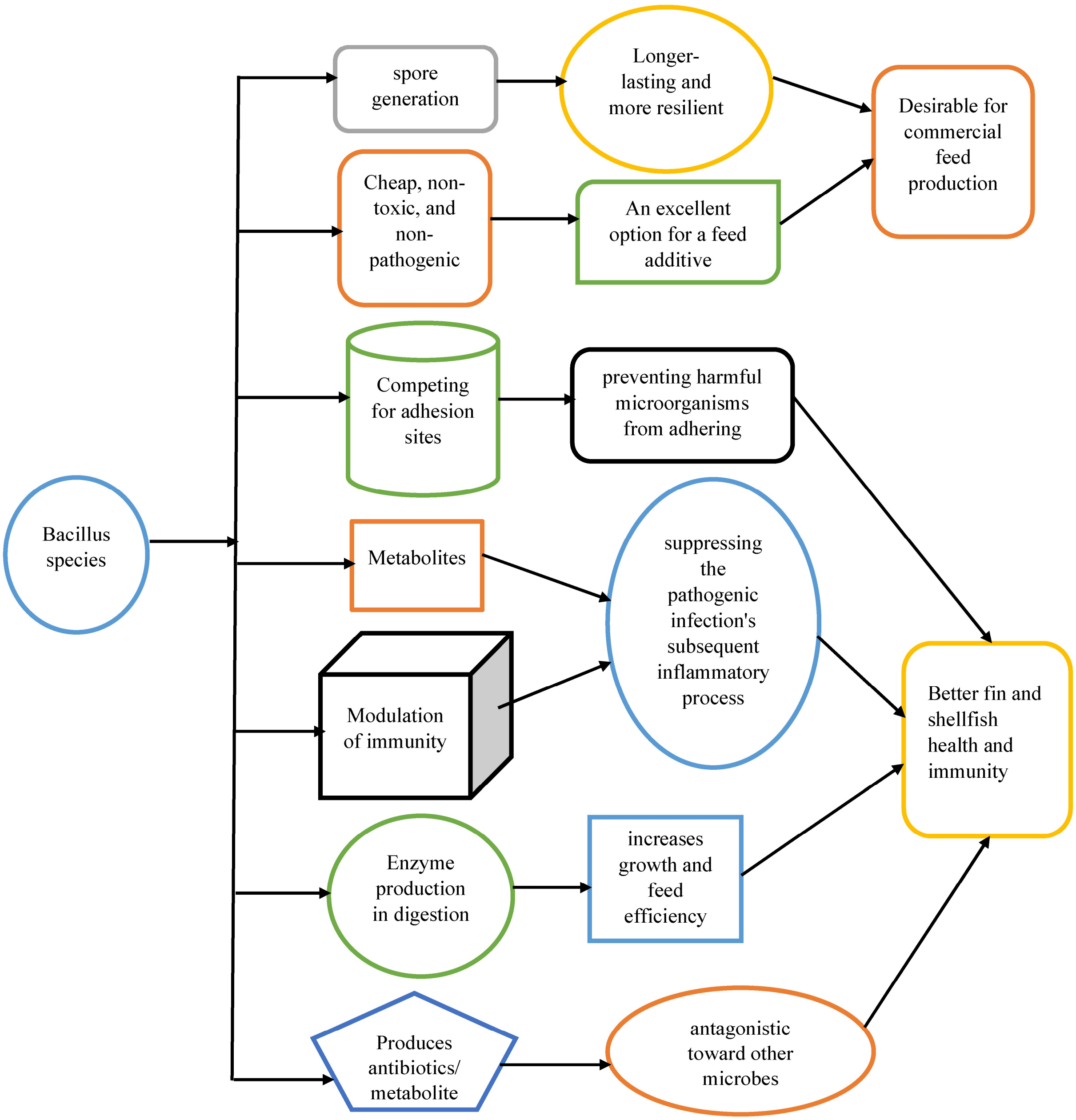

Figure 2. Features of *Bacillus* to enhance immunity and health in fin and shellfish as well as commercial production of feed. Adopted from [27].

Table 1 illustrates how some commercial *Bacillus* products have been used to generate a broad spectrum of metabolites with antimicrobial action [17]. They are employed in fin and shellfish

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production as growth boosters, disease-resistant organisms, and to improve water quality indicators [18]. Over the years, they have been used in experiments to decrease harmful bacteria in fish since they are more affordable, more effective sources of antibiotics, and sometimes even non-toxic and non-pathogenic [16,19]. Similar to other probiotics, *Bacillus* species possess characteristics such as the capacity to inhibit infections by competing for adhesion sites to halt their growth and the capacity to generate antibiotics [16,20] and bacteriocins [20], quorum quenching: the process of inhibiting the expression of virulence genes and producing lytic enzymes that break down the cell walls of pathogenic microorganisms, like cellulases, proteases, chitinases, and β-1,3-glucanases [2]. The provision of nutrients and enzymatic digestion, which promotes development by secreting digestive enzymes, is another property of *Bacillus* [4]. Additionally, the immune-stimulating properties of *Bacillus* species and their encouragement of healthy gut flora enhance the host's innate and adaptive defenses [5]. *Bacillus licheniformis*, *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilus* are the majority of the *Bacillus* species that are used as probiotics in fish [26].

Table 1. Commercial products of *Bacillus* for fin and shell fish.

Bacillus probiotic for Finfishes aquaculture		
Brand	Manufacturer	Comments
Naturalle *Bacillus subtilis*	Wuhan Nature´s Favour Bioengineering Co., Ltd, Wuhan City, China http://www.wuhannature.com	*Bacillus subtilis* (2x10^{10} CFU/g).
Biozyme	Bio-Pharmachemie Joint-Venture Company, Ho Chi Minh, Vietnam http://www.biopharmachemie.com	*Bacillus subtilis* and *Saccaromyces cerevisiae*.
Fubon *B. subtilis*	Angel Yeast Co., Ltd. Hubei, China http://www.angelyeast.com	*Bacillus subtilis* (≥ 20 billion CFU/g).
Bioron	American Pharma International, India http://www.americanpharmainternational.com	Each kg contains: *B. subtilis* (4.5x10^8 CFU), *B. licheniformis*, *B. megaterium*, *Lactobacillus lactis*, *L. helveticus*, *Nitrosomonas sp*. *Nitrobacter sp*. *Saccharomyces cerevisiae* and *Aspergillus oryzae*.
Lactomin	American Pharma International, India http://www.americanpharmainternational.com	Each kg contains: *B. subtilis* (45,000 million CFU), *B. licheniformis*, *Lactobacillus acidophilus*, *L. sporogenes* and *Saccharomyces cerevisiae*.
Bacillus probiotic for shellfishes aquaculture		
Brand	Composition	Dose
Aqua photo	*Bacillus subtilis* and *Rhodopseudomonas*	50–70 ml/100 dec. Control unwanted gas, sediment and increase growth of plankton
Bio-zyme	*Bacillus subtilis*, *Saccharomyces cerevisiae*	500 g/100 kg feed

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Eco marine	*Bacillus subtilis*, *Bacillus pumilis*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*	3–4 tablet/acre
Golden Bac	Yeast,*Bacillus subtilis*, *Lactobacillus sp*.	1.5–2 kg/acre
pH fixer	*Bacillus sp*.	1–2 kg/acre
Procon-PS	*Bacillus sp*. *Rhodococcus*, and *Rhodobacter*	5 L/hac (l m depth)
Super Biotic	*Bacillus sp*.	1–2 kg/acre

3. Utilizing *Bacillus* in Shellfish and Finfish

3.1. Application of Bacillus in Fin fishes

Numerous investigations have discovered counts of perhaps *Bacillus*, but no additional identification has been carried out. In this section, the following, bacteria identified as *B. aerophilus, B. aerius, B. amyloliquefaciens, B. aryabhattai, B. altitudinis, B. atrophaeus, B. circulans, B. cereus, B. clausii, B. flexus, B. coagulans, B. megaterium, B. stratosphericus, B. licheniformis, B. thermoamylovorans, B. methylotrophicus, B. sonorensis, B. pumilus, B. tequilensis, B. subtilis, B. thuringiensis, and Solibacillus silvestris* isolated from the finfish GI tract are discussed.

3.1.1. *Bacillus aerius*

B. aerius was proposed as a new species within the genus *Bacillus* by [28]; it was isolated from a cryogenic tube used for sampling air from high altitudes [29]. The strain was able to form endospores, stained Gram-positive, and contained peptidoglycans in its cell walls, with DL-diaminobutyric acid serving as the diamine [29]. The strain's variations in genotypic and phenotypic characteristics allowed it to be identified from strains of the closest related species [29,30]. Few research, as far as we know, have isolated this bacterium from finfish's gastrointestinal tract. [31] found autochthonous *B. aerius* in the distal intestine (DI) catla (*Catla catla*) in a study that examined the probiotic properties of exoenzyme-producing bacteria.

Another study used *B. aerius* strain B81e, which was chosen for its probiotic qualities both in vitro and in vivo after being carefully isolated from the gut of healthy catfish. This bacterium produced a substance resembling bacteriocin and inhibited both Gram-positive and Gram-negative bacteria, including the fish diseases *Aeromonas hydrophila* and *Streptococcus agalactiae*. It also demonstrated broad-spectrum antibacterial action [32].

Another study examined the positive effects of mixed probiotics (*B. aerius* B81e þ *L. paraplantarum* L34b-2) on fish growth performance, innate immunity, and illness resistance. According to the study, Pangasius fish showed notable probiotic benefits when Bacillus aerius from the Bacillus genus was combined with other bacteria [33].

3.1.2. *Bacillus aerophilus*

This species possesses traits common to *Bacillus* species: it is Gram-positive stained, capable of producing endospores, and contains DL-diaminobutyric acid-containing cell-wall peptidoglycans [12]. [28] proposed *B. aerophilus* as a new species in the genus *Bacillus* based on isolates from single strains kept in cryogenic tubes that were used to gather air samples at high elevations. [34] reported Bacillus aerophilus, a bacterium with probiotic potential based on good growth in intestinal mucus, resistance to diluted bile juice (2–20%), safety for the target fish, and production of bacteriocin in a study evaluating the autochthonous microbiota of Indian major carp (*Cirrhinus mrigala*) by cultivation.

In a different investigation, *B. aerophilus* was identified and employed to strengthen *Labeo rohita's* immune system as well as assess the impacts against *Aeromonas hydrophila* [35].

3.1.3. *Bacillus amyloliquefaciens*

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Bacillus amyloliquefaciens was initially isolated from soil in 1943 by Juichiro Fukumoto, a scientist from Japan. The species' unusual characteristic led to the naming of the species because it generated a liquefying α-amylase (amylo) [36,37].

Gram-positive, nonpathogenic, endospore-forming *B. amyloliquefaciens* is a member of a class of free-living soil bacteria that has several characteristics, such as promoting plant growth, producing metabolites that are antifungal and antibacterial, and producing industrially significant enzymes [38]. In eight finfish research involving eight different fish species, this bacterium has been isolated; in seven of those studies, culture-based techniques were employed. The majority of strains had the ability to produce bacteriocin, enzyme production and antagonism, and probiotic potential. More recently, the intestines of flounders have been used to identify this specific species of *Bacillus* [39], southern flounder (*Paralichthys lethostigma*) [40], rohu (*Labeo rohita*) [41] and Indian major carp [42] employing techniques based on culture, and these investigations showed encouraging aspects.

3.1.4. *Bacillus altitudinis*

The rod-shaped, gram-positive aerobic bacterium *B. altitudinis* belongs to the phylum Firmicutes. The first report of its isolation came from samples of extremely UV-stressed air taken in the stratosphere [28]. Since then, reports of B. *altitudinis* have been made in a variety of habitats, such as the deep freshwater of Manasbal Lake [44], the southern Indian Ocean [43], soil [45], and silt [46].

It has been reported by the culture in the DI of mrigal [47], proximal intestine (PI) of walking catfish (*Clarias batrachus*) [48] and stinging catfish (*Heteropneustes fossilis*). In a study, the bacteria *B. altitudinis* AP-MSU was isolated from the stomach of marine fish *Sardinella longiceps* and found to be capable of producing esterase at a reasonable cost by using fish processing waste [49].

3.1.5. *Bacillus aryabhattai*

Bacillus aryabhattai are widely distributed in nature but were only discovered in 2009 [50]. It has been established that this isolate is a long rod, spore-forming, motile, strictly aerobic Gram-positive bacteria that grows best at 30 °C and is non-pigmented on agar-containing media [51].

This species was found in a study that evaluated the probiotic value of bacilli isolated from the intestines of *Rhynchocypris lagowskii* [52].

Through culturing, this indigenous bacterium has been identified in the DI of mrigal [47], Walking catfish's proximal intestine (PI) [48] and stinging catfish [53].

3.1.6. *Bacillus atrophaeus*

Migula initially identified and isolated *B. atrophaeus* as *B. globigii* in 1900 [54]. After observing pigment formation when cultured in medium containing tyrosine, it was reexamined and reclassified as *B. subitilis* var niger [55]. Like other endospore-forming bacteria, *B. atrophaeus* has three distinct stages in its life cycle: vegetative growth, sporulation, and germination [56]. Spores have a remarkable resistance to environmental damages, including heat, radiation, toxic chemicals, and pH extremes, and they can remain dormant for extended periods of time [54]. Spore germination and outgrowth is the process by which a spore breaks its dormancy and resumes growth in the presence of favorable environmental conditions [54]. This indigenous bacterium has been identified through culturing in the proximal intestine (PI) of walking catfish (Clarias batrachus) [48], stinging catfish (*Heteropneustes fossilis*) [53], and the DI of mrigal [47]. The primary characteristic of these bacilli is their capacity to manufacture enzymes, and *Bacillus atrophaeus* is well-known for producing antibiotic substances [54].

3.1.7. *Bacillus cereus*

The *B. cereus* group comprises Gram-positive bacteria with low GC content, which belong to the phylum Firmicutes [12]. At least eight closely related species of rod-shaped, facultatively anaerobic, aerobic, spore-forming bacteria are included in this group: *B. anthracis, B. thuringiensis, B. cereus, B. mycoides, B. weihenstephanensis, B. pseudomycoides,B. toyonensis and B. cytotoxicus*[12]. The genomes of the *B. cereus* group species, with sizes ranging from 5.2 to 5.9 Mb and relatively comparable 16S rRNA gene sequences, are substantially conserved, with the exception of B. cytotoxicus, which is the most diverged of the group with a chromosome of 4.085 Mb (2) [12]. The mrigal PI and DI contained this amylase, cellulase, and protease-producing bacterium [21]. Cultivable native Bacillus cereus was discovered in the DI of wild olive flounder (*Paralichthys olivaceus*) fish in a study [22] evaluating the microbial diversity in the guts of farmed and wild fish. When the bacterial population in DI of Atlantic salmon (*Salmo salar*) was analyzed by culture, *B. cereus* was found to be a component of the community [57]. In a study by [42], *B. cereus* was discovered from the intestines of an Indian big carp using a culture-based approach.

3.1.8. *Bacillus circulans*

Gram-positive *B. circulans* is a rod that is motile by peritrichous flagella and has a size range of 2.0-4.2 x 0.5-0.8 μm [58]. Colonies that are cultivated at 30°C have irregular margins, an opaque cream color, and a diameter of 1-3 mm [58]. On nutrient agar, the growth is thin and can spread quickly in certain strains [58]. One type of facultative anaerobe is *B. circulans* [58]. Endospores are produced by this bacterium and can be ellipsoidal, subterminal, or terminal [59]. When living conditions are unfavorable, spores enable bacteria to lie dormant for prolonged periods of time. However, the endospore can reactivate itself into its vegetative stage when favorable conditions arise again [59].

In four culturing tests, *B. circulans* was discovered in the GI tract of rainbow trout fed different antibiotics [60]. Research on the intestinal microbiota of rohu [61], common carp [62], and mossambicus tilapia [63] also revealed thisbacteria. The strains exhibited enzyme-producing activity in the rohu, common carp, and tilapia tests.

B. circulans was isolated from *Catla catla's* intestinal tract and supplemented on *C. catla* fingerlings in a study. Supplementation had favorable effects on growth performance, immunological response, and feed utilization efficiency [64].

3.1.9. *Bacillus clausii*

Even in the presence of antibiotics, *B. clausii*, an aerobic, gram-positive, rod-shaped bacterium, can produce spores and endure passage through the stomach's acidic environment to colonize the intestine [65]. This *Bacillus* species was isolated by [66] from the gut of an orange-spotted grouper (*Epinephelus coioides*), and it showed amazing resistance to mimicking the GI environment as well as antagonistic activity against certain potentially harmful bacteria. In a different study, this species of *Bacillus* was isolated from the intestinal tract of groupers (*Epinephelus coioides*), and it showed benefits for the growth and general well-being of Japanese flounder fish when compared to other diets [67].

3.1.10. *Bacillus coagulans*

A gram-positive, spore-forming, microaerophilic bacillus that produces lactic acid is called *B. coagulans*. It was first identified as *Lactobacillus sporogenes* by Horowitz and Wlassowa in 1932 after it was isolated [68]. Based on its biochemical characteristics, the organism was reclassified in 1957 in Bergey's Manual of Determinative Bacteriology; as a result, *Bacillus coagulans* is the current correct nomenclature [69]. Among probiotics, *B. coagulans* is special because it has a protein coating that resembles a spore and protects it from stomach acid. This coating enables *B. coagulans* to enter the small intestine, germinate, and grow [68]. [21] identified autochthonous bacteria that produce amylase, cellulase, and protease in the posterior and distal intestines of three major Indian carp species: catla, mrigal, and rohu. [70] in their study isolated and identified the potential gut adherent *B. coagulans* from the stomach of *Catla catla* and to assess how supplementing that *B. coagulans* affects the experimental fish's ability to grow and retain nutrients. Common carp is the source of *B. coagulans*, which is utilized as a dietary probiotic. Growth performance, the capacity to elicit a favorable immunological response, and meat quality all showed improvements [71].

3.1.11. *Bacillus flexus*

Oxidase-positive, rod-shaped, aerobic, Gram-variable *B. flexus* is a bacterium that forms endospores [72]. The ellipsoidal endospores are found in unswollen, central/paracentral sporangia [58]. When cultured for 24–72 hours at 30 ± 2°C, it yields opaque, cream-colored, raised-margin

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colonies in the lab [58]. This bacterium species has only been identified in two investigations that looked at the GI tract of mrigal [73] and walking catfish (*Clarias batrachus*) [48].

3.1.12. *Bacillus licheniformis*

A spore-forming, Gram-positive soil bacterium is called *B. licheniformis* [58]. It can survive under favorable conditions in a vegetative state or in a dormant spore form to withstand harsh environments [58]. This species produces a variety of extracellular enzymes that may aid in the natural cycling of nutrients and is closely linked to the extensively researched model organism *B. subtilis* [58]. Numerous species of warm- and cold-water finfish have been shown to have *B. licheniformis*, varying in their ability to suppress pathogens and produce enzymes. Using culture-based techniques, [74] and [75] isolated this species of *Bacillus* from the posterior intestines of Rohu and Bata (*Labeo bata*). *B. licheniformis* was found in the posterior and distal intestines of Atlantic salmon through a culture method investigation conducted by [57]. However, the strain did not show any encouraging exo-enzyme activities or in vitro growth inhibition towards the four pathogens that were tested. Later, *B. licheniformis* was shown to be a member of the autochthonous enzyme-producing bacteria isolated from PI and DI of two species of Indian air-breathing fish, stinging catfish and murrel (*Channa punctatus*), using a standard culture approach [76]. *B. licheniformis*, an exo-enzyme-producing culturable autochthonous organism, was found in the DI of farmed olive flounder [22]. [77] discovered anomalies in exo-enzyme activity after isolating a strain of *B. licheniformis* from the mid-intestitine (MI) of long whiskers catfish (*Mystus gulio*). In three studies, *Bacillus licheniformis* was isolated from the guts of Mrigal [34], Rohu intestine [34], and Nile tilapia [78], and the results showed potential probiotic and pathogen inhibition properties.

3.1.13. *Bacillus megaterium*

Bacillus megaterium is a rod-shaped, aerobic, Gram-positive bacterium that forms spores and is found in a wide range of environments [79]. Its cells are relatively large for bacteria, measuring up to 100 μm in length and 0.1 μm in diameter [80]. Polysaccharides on the cell walls bind the cells together, which is why the cells frequently occur in pairs and chains [79]. Temperatures between 3 and 45 °C are ideal for *B. megaterium* growth, with an average of 30 °C. It was discovered that certain isolates from an Antarctic geothermal lake could grow at 63 °C [58]. [81] assessed the gut microbiota in the DI of Atlantic salmon and reported no notable features. [63] and [47] discovered that the guts of Mossambicus tilapia and PI of grass carp (*Cetenopharyngodon idellus*) were producing antagonistic *Bacillus megaterium* and exo-enzymes.

3.1.14. *Bacillus methylotrophicus*

Gram-positive *Bacillus methylotrophicus* is a rod-shaped, specifically aerobic, mobile bacterium that also produces endospores [82]. It is reported to grow well at 30°C with a pH of 7.0 in ammonium mineral salts (AMS) medium containing 600 mM methanol [82]. It was discovered by [22] that *B. methylotrophicus* is a part of the microbial diversity in the DI of wild and farmed olive flounder. Additionally, *B. methylotrophicus* was isolated from the EI of channel catfish [83] and PI of Indian major carp [84]. More recently, [41] and [85] isolated *Bacillus methylotrophicus*, which may have probiotic qualities, from the PI of rohu and the EI content of rainbow trout.

3.1.15. *Bacillus nealsonii*

Bacillus nealsonii, a spore-former belonging to the genus *Bacillus*, was isolated from a spacecraft assembly facility. It is defined by its phenotypic traits, 16S rDNA sequence analysis, and DNA-DNA hybridization studies [86]. Endospores are produced by this rod-shaped, facultatively anaerobic, gram-positive eubacterium [86]. This new species of bacteria has spores that are resistant to UV, gamma, H_2O_2, and desiccation [87]. *B. nealsonii* was discovered in the intestine of rainbow trout when antibiotics were used against both significant and non-pathogenic fish pathogens [88].

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3.1.16. *Bacillus pumilus*

A common Gram-positive spore-forming bacterium found in a variety of settings, such as soil, deep-sea sediments, and marine water, is *B. pumilus* [89]. Significant resistance to environmental stressors, such as drought, low or no nutrient availability, irradiation, UV radiation, chemical disinfectants, or oxidizing enzymes, is exhibited by this species [90]. The first study, carried out by [91], identified *B. pumilus* in rohu fish intestines. The bacterial isolate generated extracellular protease, amylase, and cellulase, leading the researchers to speculate that this specie of bacteria might be essential for rohu fingerling nutrition. [92] identified an allochthonous *B. pumilus* that may have probiotic qualities from the EI of channel catfish. [83] discovered *B. pumilus* in the gut of orange-spotted grouper. Based on cultivation, *B. pumilus* was revealed as a member of the bacterial population derived from the fish distal intestine (DI) in [93]'s study of the gut microbiota of *Salmo trutta*. Furthermore, [73] recovered this autochthonous bacilli species with chitinae-producing characteristics from the silver carp (*Hypophthalmichthys molitrix*) DI. When rainbow trout (*Onchorhynchus mykiss*) were served linseed oil, autochthonous *B. pumilus* was discovered in their diet, according to a review published in [94]. The bacteria, however, was absent in the DI of fish that were fed marine, rapeseed, or sunflower oil. From the PI of mrigal and rohu, species of the enzyme-producing *B. pumilus* exhibiting an unfavorable trait were sequestered [47]. With an excellent adhesion feature, this bacilli species was recently isolated from the EI rainbow trout by [85].

3.1.17. *Bacillus sonorensis*

Gram-positive aerobic bacterium *B. sonorensis* belongs to the *Bacillus subtilis* group of microorganisms and forms endospores [95]. It was originally isolated from soil in the Sonoran Desert, and it shares many characteristics with *B. licheniformis*, including the ability to be facultatively anaerobic, with it [96]. [97] discovered strains of antagonistic autochthonous *B. sonorensis* with the ability to produce enzymes in the DI of mrigal. The author suggested that more in vivo studies be conducted to clarify their effects on growth performance and health in light of their findings.

3.1.18. *Bacillus subtilis*

B. subtilis is a Gram-positive, catalase-positive bacterium that is found in soil, the gastrointestinal tracts of humans, ruminants, and marine sponges. It is also referred to as the hay bacillus or grass bacillus [98]. *B. subtilis* is a rod-shaped member of the genus *Bacillus* that can withstand harsh environmental conditions by forming a hard, protective endospore [30]. Despite evidence that *B. subtilis* is a facultative anaerobe, it has historically been categorized as an obligate aerobe [58]. Several studies on finfish have revealed this species in their GI tracts [99–102]. It was identified as autochthonous in Atlantic salmon PI and DI by [57], and further research revealed similar results by [81]. [103] investigated the microbial communities present in the digestive tracts of three distinct fish species. From the intestine of catfish, a *B. subtilis* strain which was isolated exhibited exceptional antimicrobial activity against *Edwardsiella ictaluri* [83]. [76] used a base-culture technique to recover *B. subtilis* via three fish species. Additionally, this sort has been derived using culture techniques from cyprinid species with DI content [104], southern flounder intestines [39], Nile tilapia PI and DI [105], and Indian major carp [42]. It is important to note that autochthonous *Bacillus subtilis* was isolated from MI of mrigal [106] and that this revealed high phytase activity. This information was then utilized in a solid-state fermentation study [107] when discussing the presence of *B. subtilis*. An autochthonous *B. subtilis* that may be a probiotic was isolated from DI Indian major carp [84]. The authors proposed that the bacterium could be a bio-control agent, but further research into in vivo studies is warranted.

3.1.19. *Bacillus tequilensis*

B. tequilensis is a rod-shaped, single-celled, motile, Gram-positive bacterium. In terms of biochemistry, *B. tequilensis* and *B. subtilis* are fairly similar; however, positive arginine hydrolases, lysine decarboxylase, ornithine decarboxylase, and the ability to produce acid from rhamnose can distinguish the two species [108]. Only one recent study has shown that this species is present in the PI of silver carp, indicating that it is rarely isolated from finfish [47]. In comparison to the other

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derived from the fish group, the isolate showed high activities of cellulase and xylanase. Furthermore, strain HMF6X exhibited anti-*Aeromonas salmonicida* activity.

3.1.20. *Bacillus thermoamylovorans*

A facultative anaerobic organism, *B. thermoamylovorans* can ferment in the absence of oxygen and produce ATP in the presence of oxygen [58]. Spores produced by *B. thermoamylovorans* have a high degree of heat resistance, and they have been shown to withstand industrial food sterilization procedures [109]. The organism can grow between 40°C and 58°C and is facultatively anaerobic [110].

To our knowledge, only one finfish study [111] investigated contents of *Salvelinus alpinus* and found *B. themoamylovorans* However, neither the probiotic potential nor the production of extracellular enzymes were further evaluated; these are topics that demand more investigation.

3.1.21. *Bacillus thuringiensis*

The entomocidal parasporal crystal proteins that *B. thuringiensis* produces, its capacity to dwell in an environment free of other Gram-positive spore-forming bacilli, and its ability to survive in an exclusive environmental niche and in the gut of organisms are just a few of its many unique characteristics [112]. [57] showing in their research a native in Atlantic salmon indicated Bacillus derived inhibited the growth of pathogens. Subsequently, [88] revealed *B. thuringiensis* in rainbow trout gut.

A study's two culture-based tests demonstrated the cellulase activity of a strain of *Leporinus friderici* that was isolated from the midintestine (MI) [113]. The probiotic ability of a native populations derived from the pisces of major carp was later demonstrated [34].

3.1.22. *Bacillus silvestris*

Round endospore-forming, rod-shaped, peritrichously flagellated, aerobic, Gram-positive bacteria is called *B. silvestris* [58]. In a swollen sporangium, the spore position is terminal [114]. Members of *Bacillus* RNA group 2 are related to it [115]. The primary structure of the 16s rDNA and its phenotypic characteristics allow the isolate to be identified from other species within this group [115]. In a study carried out by [116] the probiotic *B. silvestris* was initially isolated from forest soil. According to [115], the bacteria was reclassified as *B. silvestris*. The presence of *B. silvestris* in fish gastrointestinal tracts was studied in Indian large carp [34].

4. Effects of *Bacillus spp* on Fin Fish and Shellfish

Bacillus spp has been proven to be a viable, safe and reliable probiotic in finfish and shellfish culture [7]. It has been shown that certain *Bacillus* species increase the activity of digestive and antioxidant enzymes, improve feed utilization for faster growth, express genes related to immunity and stress, and, most importantly, strengthen the fin's defenses against harmful microorganisms [24,117,118]. This section elaborates on the effects on finfish and shellfish culture.

4.1. Impact of *Bacillus* Species on Finfish

Over the past ten years, considerable work has been done to evaluate different bacterial species as probiotics in aquaculture [119,120]. *Bacillus sp*. has a positive impact on the growth performance and disease resistance of several fish species [27,121]. It appears that *B. subtilis* was the primary focus of the investigations conducted on *Bacillus sp* [122]. The impacts of several *Bacillus* species on fin fish species are outlined and discussed in this section.

4.1.1. The Effects of Bacillus on Immunological Parameters and Disease Resistance in Finfish

Bacterial infections are the most frequent cause of disease issues in aquaculture [123]. *Streptococcus agalactiae* is one of the bacterial pathogens [124]. According to reports, and *Aeromonas hydrophila* [125] can result in significant financial losses for fish farms. Drug-resistant microorganisms have emerged posing a threat to the environment and compromising food safety [126,127]. Additionally, vaccinations cannot be used as a universal control agent because they are only effective against a limited subset of pathogenic bacteria [128]. Probiotics have therefore been presented as an alternative to improve fish health and manage illnesses [129,130]. Probiotics are thought to work by

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generating antimicrobial compounds, which drive out pathogens and compete with them for nutrients and space [131,132]. They also cause the host's nonspecific or specific immune system to become more active, which strengthens the host's defense against pathogens. An increasing amount of research has specifically addressed the application of *Bacillus* species to the management of bacterial diseases in aquaculture. It appears that after adding *Bacillus* to the diet, there was an increase in resistance to *S. iniae* [133], *A. hydrophila* [134], *Acinetobacter* sp. and *Acinetobacter tandoii* [135], as well as *Aeromonas salmonicida, S. agalactiae, Lactococcus garvieae*, and V*ibrio parahemolyticus* [136]. Additionally, it has been documented that dietary *Bacillus subtilis* administration improves disease resistance in a variety of aquatic species, including rainbow trout [137], tilapia [138], and white shrimp [139]. As a result, *Bacillus* species have proven to be useful as substitutes for antibiotics. The probiotic *Bacillus sp*. reduced the abundances of Aeromonas and Pseudomonas sp. in the tilapia gut microbiota [140]. The Nile tilapia's resistance to disease and general health were improved by *Bacillus pumilus* and *P. fluorescens* [141]. Diets enriched with *Bacillus subtilis* exhibit enhanced prophylactic qualities against the pathogenic microorganism *Streptococcus agalactiae* [142].

4.1.2. Effects of *Bacillus* as a Growth Promoter in Fin Fishes

It is now widely acknowledged that a significant amount of farmers' expenses—roughly 50–60%—are related to diet [143]. As a result, numerous researchers are developing various strategies to cut costs, particularly through the use of various growth parameter kinds [144,145]. Probiotics have exhibited growth properties in variety of fish species if administered through diets [146]. Probiotics are thought to do this by improving intestinal physiology and generating exogenous enzymes [147].

In a study conducted by [148], *B. subtilis* was fed to rohu at three different levels (0.5, 1.0, and 1.5 $\times 10^7$ CFU g−1) for a period of 15 days, and a noteworthy rise in weight gain was noted. Afterwards, [149] added different concentrations (4.8×10^8, 1.2×10^9, 2.01×10^9, 3.8×10^9, and 6.1×10^9 CFU g-1) of commercial *B. subtilis* to the feed of rainbow trout fry. Fish fed probiotics demonstrated a discernible increase in growth indices after administration (13 days). Additionally, in a 28-day feeding trial, [150] examined the effects of oral *B. subtilis* E20 (10^4, 10^6, and 10^8 CFU g-1) administration on growth performance parameters of orange-spotted grouper. The results showed that feeding efficiency and weight gain were significantly increased by dietary probiotics [150,151]. [152] fed grass carp with a different strain (*B. subtilis* Ch9). After 56 days of feeding, fish treated with varying amounts of probiotics (1.0×10^9, 3.0×10^9, and 5.0×10^9 CFU kg-1) showed significantly higher weight increase, SGR, and FCR. Additionally, the authors observed that fish administered probiotics had improved intestinal morphology and a notable increase in the activity of digestive enzymes. In a comparable way, feeding olive flounder with *B. subtilis* boosted their ultimate weight, FCR, and protein efficiency ratio much more than feeding them the control [153]. Moreover, adding 5×10^6 CFU g-1 of *B. subtilis* to the diet of Nile tilapia considerably enhanced growth performance metrics [154]. When Nile tilapia (65 5 g) were fed *B subtilis* for two months, the fish's weight gain and survival rate increased dramatically [155]. Recently, [156] revealed in a two-month supplementation of *B. subtilis* efficiently improving growth, intestinal probiotic recovery and enzyme activities in *O. niloticus*. Moreover, in feeding *Larimichthys crocea* supplemented diets of B. subtilis for averagely two months enhanced growth was observed [157]. Oral feeding of Nile tilapia for 10 weeks showed similar outcomes [158]. [158] revealed that high dosage of Bacillus improve wellbeing of fish intestines compared to the control and low dosages. However, [159] In a study with catfish (*Pangasius hypophthalmus*), smaller dosages of *B. licheniformis* were more beneficial on growth than higher dosages, showing the host and probiotic specific species [160].

Moreover, aside *B. subtilis*, investigations of other *Bacillus* species have been undertaken to evaluate effects on the growth factors of fish. [64] investigated the effects of *B. circulans* extracted from *Catla catla's* gut as a feed supplement. After a two-month feeding experiment, the scientists found that fish fed bacillus-supplemented diets had improved growth performance measures. Additionally, [161] gave a distinct dosage of dual types of Bacilli in an orange-spotted grouper diet throughout a 30-day study. Probiotic strain from orange-spotted grouper's intestines was provided by the authors. These probionts, in contrast to other research using Bacilli probiotics, were incapable of altering development metrics. On the contrary, the impact of *B. amyloliquifaciens* were assessed in a three-month diet treatment with *O. niloticus* [162]. The findings showed that *B. amyloliquifaciens* had no meaningful effect on *O. niloticus*. A two-month dietary delivery of *B. amyloliquefaciens* in turbot

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showed a moderate boost in fish growth metrics, but enzymes from the gut showed a considerable increase compared to the control fish [40]. In another study it was realized that silver carp given *B. latrospores* and *B. licheniformis* saw a superior growth performance compared to the control [163]. Moreover, a two months' oral application of bacillus improved growth of Athlantic salmon in recirculatory aquaculture [164]. In another study, the researcher observed a combined multi-species administration of *Bacillus* showed beneficial effects on fish compared to single administrations. Other research agreed [165] when *B. subtilis* in combination with *P. aeruginosa* + *L. plantarum* was employed in *Labeo* rohita with a good outcome in enhancing of growth parameters than using only *B. subtilis*. On a contrary to a study conducted [166] no significant difference was observed when Tilapia was fed a single form or combination with *B. velezensis* for a month.

Besides the various ways of administering *Bacillus* a few has studies has evaluated the effects of bath applications. For example, in an investigation with *O. niloticus*, [167] used *Bacillus coagulans* as water probiotic. After a month, the researchers found that fish in the probiotic water bath improved in their growth metrics. Furthermore, 56-day administration of B. subtilis as a water addition resulted in a significant increase in overall weight and specific growth weight in the probiotic-treated fish. [167].

Table 2. Effects of *Bacillus spp* on growth of fin fishes.

Bacillus spp	Fish species	Initial Weight	Application of *Bacillus spp*	Observation on Growth	Conclusion	References
B. subtilis	*Oreochromis niloticus*	14.82 ± 0.42 g	Dietary application for 50 days	Fish given probiotics showed noticeably improved growth results as compared to the control group, and their digestive enzyme activity also increased noticeably	In tilapia housed in a biofloc system, *B. subtilis* efficiently increases fish output, immunity, and defense against LPS-induced damages	[168]
(a mix of *B. subtilis* and *B. licheniformis*)	*Oreochromis niloticus*	53.01 ± 1.0 g	Dietary application for 50 days	All probiotic BS enriched groups showed improved weight	Application of probiotic BS at 10 gkg-1 (BS10) may be taken into consideration to enhance tilapia	[169]

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				gain, specific growth rate, and feed conversion ratio	farming growth	
B. amyloliquefaciens and *B. pumilus* isolated from striped catfish (*P. hypophthalmus*)	(*Pangasianodon hypophthalmus*)	25.2 ± 1.3 g	dietary supplementation	Growth improvement in fish given a combination of probiotics	Thus, striped catfish health and growth rate can be enhanced by dietary supplementation of a blend of *B. amyloliquefaciens* and *B. pumilus* at 5 × 10^8 CFU g−1	[170]
Two species of *Bacillus* (*B. licheniformis* and *B. subtilis*)	Asian Sea Bass, *L. calcarifer*	1.5 ± 0.2 g	During 8 weeks of dietary supplementaion	Compared to Asian sea bass fed the basal food (control), those supplemented with probiotic *Bacillus* (*B. licheniformis* and *B. subtilis*) had noticeably improved growth.	Considering that the optimal outcome is obtained when 1 ×10^6 CFU g−1 of *Bacillus* is supplemented in the diet	[160]
(*B. subtilis* and *B. licheniformis*	*Oreochromis niloticus*	mean weight of around 150 g	30 days of dietary supplementation	The fish fed with diets containing 0.04% and	The establishment of a beneficial microorganis	[102]

				0.08% of probiotics presented higher weight gain than the control group	m population may improve host health	
Mixed probiotic containing (*B. licheniformis* and *B. subtilis* and Ferroin solution)	kutum, (*Rutilus frisii kutum*)	0.4 ± 0.1 g	60 days of supplemented diets in ratios	Fish receiving diets supplemented with probiotics and Ferroin solution showed significantly better growth than those fed the basal diet (control)	These results indicate that the combination of probiotic and Ferroin solution represents an effective dietary supplement for growth performance	[171]
B. subtilis	grass carp, *Ctenopharyngodon idella*	50 ± 2.5 g	56 days dietary feeding	Significantly higher SGR and lower FCR than those fed the control diet	An optimum dose of B. subtilis Ch9 could induce digestive and potentially promote the digestion and absorption of nutrients, as well as improve the growth performance of grass carp significantly.	[152]

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B. subtilis	*Oreochromis niloticus*	16.5 ± 0.2 g	21-d growth trial as feed additives	There was no discernible difference in growth performance when any probiotic-added diet was used	Given the short study period, these outcomes are not shocking	[99]
B. pumilus	*Oreochromis niloticus*	3.62 ± 0.06 g	84 days of dietary supplementation	The study showed fish fed a pro-enzyme diet had improved feed consumption and growth performance	Pro-enzyme supplementation enhanced the growth performance	[172]
B. circulans	*Catla catla*	6.48 ± 0.43 g	Feeding of diets supplemented for 60 day	Compared to other treatments, *C. catla* given feed probiotic showed higher growth performance in terms of live weight gain and specific growth rate	The study's findings support the use of *Bacillus circulans* PB 7, a probiotic, for improved growth and appropriate nutrient use	[64]

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B. licheniformis	*triangular bream (Megalobrama terminalis)*	30.5 ± 0.5 g	8-week feeding trial,	Fish fed *B. licheniformis* showed a considerably higher growth parameters	*B. licheniformis*, either by itself or in combination, can greatly enhance triangular bream growth performance	[173]
B. clausii	*Japanese flounder Paralichthys olivaceus*	Average weight of 21 g	Dietary supplementation for 56 days	Fish fed *B. clausii* gained more weight than the control group	The growth performance and health benefits of the Japanese flounder were enhanced by *B. clausii*	[67]

4.1.3. Bacillus spp Effects on Liver Health of Fin Fishes

Recently, there has been speculation that the accumulation of pollutants may be the reason behind gall syndrome and liver enlargement in many farmed fish [174]. A rise in the levels of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT), which interconvert amino acids with other metabolic intermediates and are involved in a variety of biochemical events in metabolism, can be an indication of tissue damage, as in the case of chronic liver disease [175,176]. According to [177], AST and ALT are sensitive biomarkers used in the detection of liver damage because they are cytoplasmic in origin and are released into circulation (blood) after cellular injury. In vertebrates, AST is present in both mitochondrial and cytoplasmic forms; the tissues containing the greatest amounts of AST are the heart, liver, muscle, and kidney, in that order [175]. Fish rely heavily on the activity of the enzymes AST and ALT because they can be used to identify tissue damage caused by toxicants (found in feed given or in the environment) [174]. *Bacillus spp*. have been found to influence AST and ALT in fish; fish fed a diet enriched with probiotic *B. licheniformis* and *B. subtilis*, for example, had decreased AST and ALT [142]. On the contrary, in a study [178], no changes were estimated in the levels of glucose, hepatic (alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and renal biomarkers (Urea, Creatinine, and Uric acid) in the serum of fish-fed bacillus supplemented diets. In another research [179], assessing the effects of *bacillus* on nile tilapia it was observed after 12 weeks that levels of aspartate aminotransferase and alanine aminotransferase reduced compared to the control. After challenge test, aspartate transaminase (AST), alanine transaminase (ALT) activity, and liver malondialdehyde level increased significantly in control groups; however, level of these parameters were considerably lower in fish fed with probiotic supplemented diets when *B. amyloliquefaciens* CCF7 was added to *L. rohita* [180].

4.1.4. Bacillus Species-Related Effects on Finfish Gene Expression

It has been reported that certain *Bacillus* species increase the expression of genes linked to growth metabolism, inflammation, digestion, the cytoskeleton, transport of proteins, junction complex protein encoding genes, and antioxidant genes [181,182]. There aren't as many studies on *Bacillus* and the expression of the aforementioned genes as there are on the bacteria's role in regulating growth, feed utilization, etc. [183]. By reducing the expression of the ++HSP70 gene, a combination of *Bacillus* species has been shown to lessen the severity of cellular stress in sea bream larvae, improving the fish's tolerance to rearing conditions [184]. In a different study, it was found that fish fed *Bacillus* diets had significantly higher gene expression levels of heat shock protein 70 (HSP70), interleukin (IL-1β), interferon-gamma (IFN-γ), and tumour necrosis factor (TNF-α) than fish fed the control diet [100]. The liver tissue showed significant modulation of growth-related genes, such as insulin-like growth factor genes (IGF-1 and IGF-2) and growth hormone receptor genes (GHR-1 and GHR-2) in an investigation on the potential of *Bacillus* on Tilapia. The IGF-1 gene was significantly more expressed in muscle tissue [185]. Other evidence exists, including the altered expression of mucosal genes in gilthead Sea breams [186], immune-related genes expressed in the head kidney of *Carassius auratus* [187], pro-inflammatory cytokines (IL-8 and IL-1β), TLR5, and TGF-β1 expressed in the intestine and head kidney of *E. coioides* [188], and pro-inflammatory cytokines expressed in the intestine of Nile tilapia [100] following probiotic *Bacillus* administration.

4.2. Effects of *Bacillus spp* on Shellfish

Bacillus is one of the many potential probiotics in shrimp aquaculture that has produced positive outcomes [189]. *Bacillus* has been utilized to boost immunological response, treat illness, and increase growth performance [156,190]. Furthermore, it's known that *Bacillus* species secrete a variety of extracellular materials and antimicrobial peptides that enhance water quality, aid in the digestion and absorption of feed, strengthen shrimp immunity, encourage growth and reproduction, and increase shrimp's ability to withstand pathogenic microorganisms [191–194]. This section explains in detail how *Bacillus*, a probiotic, affects disease resistance, immune responses, and growth performance in cultured shrimp.

4.2.1. Enhancement of Immune Response Resistance to Diseases in Shell Fishes

The invertebrate's only defense against harmful pathogens is its non-specific immune system, which has been shown to be enhanced by probiotics [195]. According to [196], Bacillus sp. (strain S11) can be used to protect tiger shrimp (*P. monodon*) from disease by stimulating their humoral and cellular immune systems. Transduction signaling molecules produced by *Bacillus* can warn the immune system about pathogenic agent attacks [197] and certain diseases like edema of the gut [198]. Numerous research studies have demonstrated that probiotics, whether commercial or derived from other sources, can enhance the cellular and humoral components of the innate immune system in a variety of fish and shellfish species [196,197,199–206].

Probiotics that increase shrimp immunity to diseases have garnered a lot of attention during the last ten years [18,207]. It is believed that *Bacillus* species are harmless probiotic bacteria that improve the wellbeing of their hosts by boosting natural defenses and strengthening the body's defenses against harmful microbial infections [16,27]. Prior research utilizing *Bacillus* indicated that the probiotic bacteria could augment and confer resistance in shrimp species (*P. monodon*) upon encountering *V. harveyi* [208]. But the mode of action wasn't fully understood until a few authors began explaining the underlying mechanism, which included the immune response and whether or not invertebrates, like shrimps, could mount an immune response that shared some characteristics with vertebrates' immune systems. [196] noted that *Bacillus* probiotics, such as *Bacillus* S11, enhance survival and disease resistance against *V. harveyi* by inducing phenoloxidase, phagocytosis, and antimicrobial activity in *P. monodon* hemolymph, thereby stimulating the immune response. Furthermore, it has been observed that diets supplemented with *Bacillus* to shrimp species enhanced the natural defensive system, increasing their resistance to infections [209]. For example, it has been reported that *B. subtilis* E20 increases the resistance of white shrimp (*L. vannamei*) to *V. alginolyticus* by increasing phenoloxidase and phagocytic activity. Additionally, the administering of *Bacillus* to white leg shrimp enhances the immune response (phagocytosis, phenoloxidase, etc.) shielding this specie against diseases (WSSV) [210,211]. The amount of Vibrio and total viable counts of bacteria in the shrimp gut were reduced when *L. vannamei* was fed *Bacillus* probiotics [211]. *B.fusiformis* also administered to *L. vannamei* daily or at intervals increased survival and prevented disease as immune system was boosted.[212]. On the other hand, *B. subtilis* increased *Litopenaeus vannamei's* antioxidant capacity and phenoloxidase activities [213]. Numerous immunological factors, such as lysozyme, respiratory burst, phenoloxidase, and bactericidal activity in *L. vanamei*, were increased by *B. cereus*

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[214]. The improved immune status was validated by the increased survival rate of the treated shrimp following *V. harveyi* challenge. Similar to this, feed supplemented with *Bacillus spp*. at 1×10^7 and 1×10^9 CFU/kg diet and fed continuously to shrimp for 5 weeks effectively enhanced growth, as demonstrated by feed conversion ratios, average daily growth, final weight gain, and specific growth rates. Furthermore, this probiotic improved the expression of the prophenoloxidase, lysozyme, and anti-lipopolysaccharide factor genes and markedly increased immune responses through phagocytic activity and clearance efficiency [209].

A two-month trial was conducted to assess the impact of different feed probiotic supplements on young *L. vannamei*. The lysozyme activity of shrimp fed probiotics and over-the-counter diets demonstrated significant improvement when compared to the control diet. Furthermore, compared to shrimp fed control and over-the-counter diets, immune-related gene expression in shrimp fed BS8, PP8, and LL8 diets was found to be significantly higher [215].

In addition to reducing Vibrio population abundance, *B. aryabhattai* supplementation to Pacific white shrimp (*Litopenaeus vannamei*) also changed the bacterial community within the shrimp's gastrointestinal tract. *B. aryabhattai* was found to activate antioxidant and innate immune responses in shrimp [216]. Furthermore, *B. subtilis* E20 has been shown to enhance a number of immune response factors, in addition to survivability and resilience to stress in white shrimp, which includes salinity, temperature, and nitrite-N [217].

When *L. vannamei* was fed *B. subtilis* orally, it was observed that they were more resilient to ammonia oxygen shortage and had a higher survival rate than the control group [218]. This could be because the *B. subtilis* probiotic converted and transformed ammonia. [219] assessed how *Bacillus* protected juvenile *Marsupenaeus japonicus* against temperature stress by acting as an immunomodulator and protective agent. In a different investigation, demonstrating that adding *Bacillus* (such as *B. subtilis* and *B. licheniformis*) to *L. vannamei* culture water improved immune response, as evidenced by the increase in prophenoloxidase (ProPO), peroxinectin (PE), and other markers [220,221]. Additionally, probiotic bacteria increase juvenile white shrimps' resistance to disease from the pathogenic *V. harveyi* [220,222]. A strain of *Bacillus*, isolated from the gut of *Fenneropenaeus chinensis*, was shown in 2016 by [192] to enhance defense mechanisms, protecting *L. vannamei* against infection by the white spot syndrome virus. Some authors have reported an intriguing discovery: Some few species of *Bacillus* have been found to contain PHB helps aquatic animals by boosting their immune systems, both specific and non-specific [223,224]. Additionally, a small number of researchers indicated *Bacillus* for giving immune abilities to shrimp species against pathogenic microorganisms [225]. According to [193], *P. mondon* postlarvae can be stimulated to mount an innate immune response by a *Bacillus* strain containing PHB. More recently, shrimp fed *Bacillus* alone or in combination, for 21 days at 28ºC have been shown to improve the natural defense and stimulate appetite in *V. harveyi* [226].

4.2.2. Modifying Digestive Enzymes and Encouraging Shellfish Development

It has been discovered that *Bacillus* species offer encouraging development and survival in a system for raising *L. vannamei* shrimp without water exchange [227]. For aquaculture species, probiotics can therefore be thought of as growth promoters in addition to their many other advantages [7,228].

Probiotic-based microbiota manipulation in shrimp aquaculture has been shown to be an effective strategy for controlling or inhibiting pathogenic bacteria, enhancing host immune response to pathogenic infection or physical stress, and improving growth performance and digestive enzyme activity [215,229]. One anticipated benefit of *Bacillus* as probiotics is that it directly promotes development by inducing digestive enzymes, such as amylase and protease, which in turn increases the host's natural digestive enzyme activity [24,230]. It has been noted that *Bacillus sp*. improves *P. monodon* postlarvae growth and survival without requiring water exchange, and it helps the host feed shrimp, especially by supplying fatty acids and vitamins [191,197,231]. *Bacillus* probiotics are said to enhance the growth of shrimp and the activity of digestive enzymes [189,220]. The administration of *B. subtilis* to *L. vannamei* was found to enhance the activity of the digestive enzymes protease and amylase, which in turn promotes the growth of juvenile shrimp [189,220]. [232] and [221] evaluated the impact of commercial *Bacillus* probiotics on *L. vannamei* rearing performance in terms of growth, bacterial count, feed efficiency, and body composition. According to the study, *L. vannamei*

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experimental tanks supplemented with *Bacillus* had a much greater influence on growth than the control group. Furthermore, the probiotic facilitated an increase in the amount of feed that *L. vannamei* postlarvae consumed [233]. It was also reported that the addition of *Bacillus* to in white shrimp (*L. vannamei*) water improved growth features significantly. Moreover, isolated bacteria from shrimp showed promising effects on growth of *L. vannamei* [234] and [192]. The outcomes demonstrated that *Bacillus* probiotics enhanced growth performance and decreased the risks associated with stressful conditions or factors in shrimp culture. Probiotic species selection is a significant factor, as evidenced by a study by [235] that found that *B. subtilis* given to *L. vannamei* orally outgrew both non probiotic diets and *B. megaterium* groups in terms of weight gain and food-conversion ratio. When applied through diets alone or in combination, both species of *Bacillus* improved the growth of shrimp and improved conditions of their environment [226].

Under ideal circumstances, *Bacillus*, which are frequently obtained from shrimp has been demonstrated to hoard PHB in the range of 11% to more than 50% on cell dry weight [236]. *Bacillus* bacteria isolated shrimp culture ponds has been seen to hoard PHB which improves the growth and reducing stress in *P. monodon* [225].

Table 3. Effects of *Bacillus spp* on growth of Shell fishes.

Bacillus spp	Fish species	Initial Weight	Application of *Bacillus spp*	Observation on Growth	Conclusion	References
B. cereus	*L. vannamei*	4.55 g	28 days dietary administration	Findings demonstrated that following a 28-day probiotic feeding regimen, shrimp growth was enhanced in comparison to the control group	The study showed that probiotics might be used to enhance white shrimp growth	[237]
B. licheniformis	*Haliotis discus hannai Ino*	4.17 ± 0.32	8-week culture experiment	In comparison to the control group, the supplemented diet group exhibited a significantl	The study suggests *B. licheniformis* diets enhanced abalones food intake and growth.	[238]

				y greater specific growth rate of shell length, food intake, and food conversion rate		
B. amyloliquefaciens	*H. discus hannai*	4.28 ± 0.23 g	8-week culture experiment	Diets supplemented with *Bacillus* showed significantly increased body weight, specific growth rate, and food conversion efficiency compared to the control group	The experimental meal that contained 10^5 CFU/g of *B. amyloliquefaciens* promoted abalone growth and food intake	[239]
B. subtilis	(*L. vannamei*)	12.03 ± 2.76 g	1 month feeding of supplemented diets	Effective enhancement of growth	A notable rise in growth metrics suggested that *Bacillus* was the best option	[209]
B. subtilis and *B. licheniformis*	(*L. vannamei*)	1 ± 0.1 g	Dietary supplementation for a month	Shrimp's specific growth rate was much higher in the	Shrimp growth parameters were effectively increased by	[240]

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				supplemented diet than in the (control) treatment, and the treated groups' ultimate growth was comparable	the *Bacilli* probiotic combination	
B. licheniformis	*H. discus hannai*	4.91 ± 0.34 g	70 days of beimg fed dietary supplemented diets	The abalone in the supplemented diet exhibited a considerably greater feed conversion efficiency and specific growth rate compared to the control group	Maximum growth benefits of the probiotic were noted	[241]
B. subtilis	(*L. vannamei*)	0.67 ± 0.06g	Dietary application for 8 weeks	The final weight, weight gain, and digestive enzyme activity of the shrimp fed *Bacillus* diets were significantly higher than those of the	*B. subtilis* treatment can enhance shrimp growth performance	[189]

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				untreated control group		
B. cereus	(*P. monodon*)	0.204 ± 0.004 g	Dietary appliacation for 90 days	The group that received a supplemented meals achieved a higher FCR of 1.27 ± 0.081, a maximum production of 10.45 ± 0.275 g, and an SGR of 4.40 ± 0.179%, according to the overall growth responses	*B. cereus*, a lyophilized probiotic, was effective in boosting shrimp growth when added to feed at a concentration of 0.4%/100 g	[191]
B. pumilus	(*Macrobrachium rosenbergii*)	1.81 ± 0.01 g	2 months dietary application	The experimental groups exhibited significantly increased final weight, weight gain rate (WGR), and specific growth rate (SGR) compared to the control group	Adding *B. pumilus* to feed at a dosage of 1 × 10^8 CFU/g would enhance *M. rosenbergii's* growth and digestive enzymes	[242]

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4.2.3. Enhancing Shrimp Culture Water Quality Factors

In a study, [243] showed that using native *Bacillus* species in the pond waters used for *P. monodon* culture rearing could preserve the water quality and reduce Vibrio count. When [244] examined the impact two available products of bacillus on *P. monodon*, they found that ponds treated with a combination of Sacchromyces and *Bacillus spp.* exhibited relatively lower concentrations of biological oxygen demand, TAN, nitrate, and COD in beginning stages when compared to groups treated with other additives. Utilizing a blend of probiotics including *Bacillus* enhanced density of advantageous bacteria, dissolved oxygen content, and water transparency in the existing water environment of *L. vannamei* ponds. However, it decreased the levels of total inorganic nitrogen, phosphate, and COD [245]. Additionally, the pH value of the probiotic-treated group did not considerably change, but the control group's pH level significantly increased following a notable decline during the trial's first 40 days. Additionally, the application of *Bacillus* greatly increased dissolved oxygen while lowering COD [246,247]. When [248] added a *Bacillus sp.* probiotic to shrimp pond aerators, they observed a decrease in COD along with an increase in final production. In hatcheries of *P. monodon* and *L. vannamei*, the use of a combined strains of commercial *Bacillus*, was able to lower the density of Vibrio bacteria in the water column and improve the water quality [249]. In a study by [250], *M. resenbergii* was cultured and *Bacillus* was employed to treat the water. The results showed lower values of ammonia, nitrite, and pH over the course of the 60-day cultivation period when compared to the control. The ability of some native strains of *Bacillus* to reduce TAN by bioremediation was assessed after they were extracted from sea water and soil samples [231]. Additionally, *B. pumilus* was assessed on *L. vannamei* in a recirculatory system and it was realized phytoplankton and bacterial concentration was regulated [251]. The once-weekly application of an indigenous probiotic *B. subtilis* improved the water quality values two days after the bacterial community of *L. vannamei* culture was raised. As a result, there was an increase in COD and chlorophyll density and a decrease in pH, nitrite, water transparency, and soluble reactive phosphorus [252]. The pH, ammonia, and nitrite levels of the water are improved when encapsulated probiotics are added to the water column. This appears to favor the good bacteria present in shrimp and culture water. This was corroborated by [232], who found that when two mixtures of *Bacillus* species were used in the rearing water of *L. vannamei* as microencapsulated probiotics using Artemia, the levels of pH, ammonia, and nitrite in the treated shrimp were lower than in the controls. Moreover, compared to controls, the treated groups' shrimp and water had greater densities of *Bacillus* count and total heterotrophic bacteria. Additionally, some water parameters (hardness, pH and Alkalinity) increased with a reduction in other parameters when combined strains of *Bacillus* was added into water of *L. vannamei*. [220] in his study showed incorporating *Bacillus* resulted in significant improvement in water quality parameters. These parameters included salinity, bicarbonates, carbonates, Mg hardness, total alkalinity, total hardness, Ca hardness, pH and ammonia [227].

Biological methods are widely used to reduce the amount of harmful chemicals in fish farming, and the use of certain bacteria, like *Bacillus*, has been shown to be helpful in the process of converting organic wastes that are hazardous to the environment into compounds that are safe for human consumption [254]. By offering an ideal environment, *Bacillus* probiotics can improve the rearing water quality parameters [255]. This would improve the health status of aquatic animals as their environment have massive impacts on their well-being. Additionally, through bacterial competition, *Bacillus* probiotics reduce the density of potentially harmful microbes, reducing stress in culture conditions and enhancing the immunological-physiological balance of animals [6]. It is important to note, nevertheless, that *Bacillus* species are not particularly effective at removing ammonia, and no *Bacillus* strain has ever been shown to remove more than 90% of ammonia [256,257]. Therefore, a nitrifying probiotic could be a mix of a high cleaner ammonia bacterium and a *Bacillus* species could be employed to remove ammonia and nitrite from aquaculture rearing waters at the same time, as [226] demonstrated a blend of *Nitrosomonas sp*. and *Bacillus sp*. proved most effective in eliminating large amounts of TAN. A three weeks' oral use of *B. licheniformis* and *B. flexus* on *L. vannamei* showed a substantial decrease in some water parameters in shrimp rearing water, suggesting that oral application may improve the water quality conditions, even though the majority of researchers incorporating *Bacillus* did not measure the parameters of water [226].

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5. Pathogenic *Bacillus* in Fin and Shellfish

There are few studies on how *Bacillus* pathogenesis affects fish and shellfish [258]. *B. mycoides* was found by [259] to be the cause of a superficial epizootic disease in commercial channel catfish in Alabama, USA. The dorsal surface of the infected fish showed pale patches or ulcers, focal necrosis of the epaxial muscle, and chains of Gram-positive bacilli that were determined to be *B. mycoides*. Upon administering 1.6 × 10^4 cfu/fish of the isolated bacterium subcutaneously or intramuscularly to healthy catfish, lesions resembling those observed in a spontaneous epizootic occurred. The toxins produced by these bacilli bacteria could be the reason for the fish's congestion and lack of bleeding. In 2000, [260] discovered a novel bacterial white spot syndrome in Malaysian shrimp farming that was brought on by *B. subtilis* in cultivated tiger shrimp. The infected shrimp exhibited white spots resembling those of the white spot viral disease (WSVD), but they continued to grow and were active, with no appreciable death or disability. Evaluations using microscopy showed that the epicuticle's cuticle had degenerated and become discolored, and that underlying cuticular layers had been found. It was proposed that frequent use of *B. subtilis* probiotics in shrimp ponds could be linked to this kind of illness. Enzymes of protease, amylase, glucanase, and lipase can be excreted by *B. subtilis* [261] indicating the probiotics ability to cause dissolution in the shrimp epidermis makeup [262].

In an experimental study by [263] expression of *B. cereus* hemolysin II in *B. subtilis* rendered the bacterium being pathogenic for the crustacean *Daphnia magna* when it was challenged with the expressed *B. subtilis* at 04-10^6 cfu L-1 at water temperature 20 ± 5°C. The lethal concentrations 50% (LC50) on the fifth day of the experiment for the expressed *B. subtilis* and *B. cereus* were 5.4 × 10^5 and 4.5 × 10^5 cfu mL-1, respectively.

More recently, in 2015, reports of a novel bacterial white patch disease caused by *B. cereus* were received from various *L. vannamei* aquaculture sites in India [264]. The disease resulted in a persistent morbidity and mortality rate. The afflicted shrimps displayed necrosis, pale white muscles, whitish blue pigmentation, loss of appetite, and white opaque spots in their carapace. It can also release enzymes such as lipase, glucanase, amylase, and protease [265], Therefore, it could potentially be able to enter inhibited aquatic organisms under severe environmental conditions that are conducive to expressing the bacterium's virulence genes [266].

6. Fish and Shellfish Safety with Bacillus

The possible use of Bacillus as probiotics in aquatic animal feed or as a supplement to rearing water that enters the human food chain is a significant public health concern [16]. However, there is currently no information on the potential for Bacillus used in aquatic animals to contaminate food intended for human consumption [267]. The spread of antibiotic resistance brought on by transmissible antibiotic resistance genes present in certain probiotic bacteria is one of the main risk factors [268]. AFurthermore, the risks of probiotic infections and the existence of enterotoxins and emetic toxins in probiotic bacteria must be closely monitored by the aquaculture sector [269]. The majority of published information on *Bacillus* supplementation in fish farming, however, relates to their effectiveness rather than their safety [270]. Furthermore, it's critical to remember that safety evaluations and data regarding a specific Bacillus strain probiotic shouldn't be compared to those of similar bacterial strains; rather, each probiotic's safety and risk assessment should be assessed separately [271]. The extent to which a particular probiotic can be harmful depends on how susceptible the target aquatic animal-such as freshly hatched fish larvae-is to immune-physiological conditions [272]. As a result, it's possible that a probiotic strain of *Bacillus* that is deemed harmless in some circumstances is harmful in others. It is possible to claim that, similar to antibiotics, there is no particular probiotic that is 100% safe [273].

Another significant safety and quality concern with probiotics is the potential presence of undesirable or contaminated microorganisms or the harmful compounds they produce. Occasionally, these contaminants could pose a greater risk than the particular quality of the probiotics [274]. Probiotics, including those found in *Bacillus* species, are currently regarded as safe when added to aquatic animal feed or water cultures in recent reports [27,228,275]. Nevertheless, some bacterial probiotic species increase the likelihood that certain pathogenic bacteria may develop antibiotic resistance or that their enterotoxins will be produced [276].

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7. Conclusions and Future Perspectives

It is concerning how important *Bacillus* is to the rearing of fin and shellfish. This summary makes it clear that *Bacillus* has a lot of potential to support the continued cultivating of fish and shellfish by preserving the overall health of cultured fish, which includes improving growth, feed utilization, immune response, defense against infections, specifically bacterial infections, and water quality. But in order to advance studies and the use of probiotic *Bacillus* in fin and shellfish culture, we recommend the following. Future studies on the effects of probiotics should include high throughput assays for transcriptome and proteome analysis in addition to the several genome-sequencing technologies already in use. Furthermore, a detailed documentation of the transcriptome and proteome profiling of the gut microbiota is necessary to understand the various mechanisms of action of various probiotic organisms. Therefore, future research on probiotics should be highly prioritized, and a standard criterion for evaluating their impact on fish nutrition and health should include analysis at the molecular level.

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