Bacillus spp. in Aquaculture - Mechanisms and Applications: An Update View

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Abstract

Probiotics have been widely applied in aquaculture industry as sustainable and environmentally friendly tools to sustain host's health and the well-being. Among probiotics, *Bacillus* species have great potential applications in aquaculture because they can form the spores that makes them able to survive in the harsh environmental conditions. Moreover, they are nonpathogenic and nontoxic to aquacultural environments and animals. In addition, *Bacillus* species are able to produce antimicrobial substances making them more suitable candidates compared to other probiotics. In this chapter, we discussed the role of *Bacillus* in sustainable aquaculture as alternative strategies to enhance growth performance, disease resistance, and immune response of different aquaculture farmed animals.

Keywords

Bacillus · Aquaculture · Probiotics · Disease resistance

1.1 Introduction

Aquaculture is one of the world's fastest growing food sectors (Willer and Aldridge 2019). It is necessary to meet the global seafood demand, which is being accountable for 50% of the world's seafood consumption (Gómez et al. 2019). However, sustainable development of aquaculture industry is constantly defeated by the outbreak of diseases, which is considered as main obstacles to the economical

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N. Mojgani, M. Dadar (eds.), *Probiotic Bacteria and Postbiotic Metabolites: Role in Animal and Human Health*, Microorganisms for Sustainability 2,
https://doi.org/10.1007/978-981-16-0223-8_1

profitability of the industry (FAO 2020). The outbreak of diseases is also linked to application of antibiotics, posing a significant danger to the public health (World Health Organization 2014). Thus, new and natural alternatives that prohibit the incidence of diseases and improve human and animal health are urgently needed. The use of probiotics, "live organisms that can give a health benefit to the host when administered in the appropriate amounts," is a potential alternative to boost the global health (FAO/WHO 2001). The scientific community has been searching for the environmentally friendly solutions to prevent aquacultural disease, where probiotics emerged as crucial alternative to antibiotics due to advert effects of antibiotics, such as the modulation of microbiota in the aquaculture systems and the development of resistance bacteria (Kuebutornye et al. 2019; Resende et al. 2012; Ringø 2020; Wang et al. 2019a). Consequently, wide range of probiotics, such as *Bacillus, Enterococcus, Lactobacillus, Lactococcus, Micrococcus, Pediococcus, Enterobacter, Vibrio, Pseudomonas, Rhodopseudomonas, Roseobacter*, and *Shewanella*, have been found and applied to improve growth performance, immune response, and disease resistance of farmed fish and shellfish (Abd El-Rhman et al. 2009; Adel et al. 2017; Feng et al. 2019; Kuebutornye et al. 2019; Li et al. 2006; Li et al. 2020; Ringø 2020; Yang et al. 2019). In aquaculture, probiotics have been applied as functional feed additives to boost host's health and well-being via increasing growth, supplying nutrient, modulation gut microbiota, enhancing immunity, improving feed efficiency, increasing digestive enzyme activities and digestibility, and controlling diseases (Kuebutornye et al. 2019; Ringø 2020; Selim and Reda 2015).

Bacillus species are one of the most commonly used probiotics in the aquaculture industry because of their ability to form endospores, which is a benefit for industrial applications without losing their characteristics (Hong et al. 2005; Kuebutornye et al. 2019; Cutting 2011; Hai 2015). In addition, *Bacillus* is known to generate natural antimicrobial compounds, which are able to prohibit the proliferation of harmful bacteria in the aquaculture systems and host's intestines (Abriouel et al. 2011; Caulier et al. 2019; Sumi et al. 2015). Similarly, *Bacillus* species are known to stimulate the digestive enzymes, antioxidant enzymes, relative immune gene expression, and stress-related genes, which in turn improve disease resistance of the host against pathogenic bacteria (Elshaghabee et al. 2017; Nayak 2010; Soltani et al. 2019). *Bacillus* species also increase the use of feed in fish, contributing to better growth rates (Mukherjee et al. 2019; Nair et al. 2020; Xia et al. 2020). Therefore, these chapters gather recent data on the role of *Bacillus* species in promoting growth performance, disease resistance, and immune response in aquaculture.

1.2 Mode of Action of Probiotics in Aquaculture

Probiotics can affect the host's immune responses, as well as the interrelationship between these responses and their gastrointestinal microflora (Hemarajata and Versalovic 2013; La Fata et al. 2018; Yan and Polk 2011). Over the past decades, extensive researches on probiotics have provided insight into the significance of probiotics and their modes of action and numerous mechanisms have been suggested (Santacroce et al. 2019; Shi et al. 2016).

1. Probiotics improve feed efficiency and growth rate of farmed fish and shellfish (Ringø 2020; Romano 2021). They also enhance the host's appetite and feed digestion via decomposition of indigestible components, enhance vitamin productions, and detox diet's substances (Ashaolu 2020; Cencic and Chingwaru 2010; Hoseinifar et al. 2018).
2. Probiotics could compete the exclusion of gastrointestinal harmful bacteria via the secretion of peroxide, bacteriocin, siderophore, and lysozyme enzymes (Vieco-Saiz et al. 2019; Yang et al. 2014). The physiological and immunological effects are considered as one of the most essential modes of action of probiotics (Klaenhammer et al. 2012; Plaza-Diaz et al. 2019; Vieco-Saiz et al. 2019).
3. Probiotics could enhance aquaculture animal's disease resistance to stress caused by various environmental threats during aquaculture activities (Hlordzi et al. 2020; Mohapatra et al. 2013; Reverter et al. 2020).

These mechanisms display the favorable impacts of probiotics in farmed fish and shellfish. Future studies, however, on the relationship between probiotics and hosts, including metagenomics and proteomic studies, is important to clarify mode of action of probiotics.

1.3 *Bacillus* Applications in Aquaculture

1.3.1 Improve Growth Performance

The utmost target of aquaculture practice is to acquire the rapidest growth and lowest production cost. To achieve this goal, several means have been established to boost growth rate and feed consumption by adding functional feed additives and growth natural growth promoters (Hernández et al. 2016; Katya et al. 2014). Probiotics are potential tools to maintain the normal growth, health, and well-being of farmed fish and shellfish because they serve as nutrients source, vitamins, and digestive enzymes. These substances for their part will contribute significantly on feed consumption, nutrients uptake, and host's growth rate (Lauriano et al. 2016; Nath et al. 2019). Probiotics consumption have been speculated to improve the host's appetite or boost organisms' digestibility (Irianto and Austin 2002). Probiotics can improve feed efficiency of fish and shellfish by stimulating the excretion of digestive enzymes and maintaining the balance of intestinal microbes, which lead to the improvement of nutrients absorption and utilization, as well as the survival and growth of the host (Ibrahem 2015; Irianto and Austin 2002). Studies on diets containing probiotics revealed the possible involvement of these probiotics on the improvement of intestinal microflora balance and the production of extracellular enzymes to elevate the feed efficiency and growth of cultured species as growth promoters (Giri et al. 2013; Ringø et al. 2018). Most of the studies using *Bacillus* in aquaculture focus on growth performance and survival rate (Table 1.1).

1.3.1.1 Tilapias

During past decades, *Bacillus* spp. have been intensively applied in Nile tilapia aquaculture. Han et al. (2015) indicated that 10 weeks feeding trial with *B. licheniformis* significantly enhanced growth performance. However, there were no significant discrepancies in survival rate and feed conversion ratio (FCR) and in villi length and muscular layer thickness of anterior intestine among the treatments. In contrast, Iwashita et al. (2015) reveal that administration of the probiotic had no significant effect on the growth rates of Nile tilapias, although the fish fed probiotics had better feed conversion. Likewise, no significant difference in growth performance and FCR was observed in Nile tilapia fed *Bacillus amyloliquefaciens* (Silva et al. 2015). This can be explained due to the low temperatures during experimental period. Marcusso et al. (2015) reported that the homeostasis of Nile tilapia rearing at temperatures below 24 °C could be affected, enhancing the susceptibility to bacterial infections and impairing the growth performance. No effects were observed on the growth performance of Nile tilapia fed *Bacillus subtilis* (Aqua NZ and AP193) and *Bacillus subtilis* strains (Addo et al. 2017a, 2017b). These results are not unexpected given the short duration of this trial. This statement agrees with Apún-Molina et al. (2009) who observed a tendency toward improved growth in Nile tilapia fry (0.14 g) only after 75 d of feeding with diets composed of *Bacillus* or *Lactobacillus* probiotics. On the contrary, dietary inclusion of *Bacillus subtilis* significantly improved body weight, percent weight gain, specific growth rate, and feed conversion ratio (Liu et al. 2017). It is well documented that *Bacillus* exoenzymes are very efficient at metabolizing a large variety of carbohydrate, lipids, and proteins (Liu et al. 2009). The exoenzymatic activity of *Bacillus* spp. is one of the main reasons for its ability to improve digestive enzyme activities (Han et al. 2015). Higher enzyme activities in the digestive tract enhance digestive capability and growth performance of the host. It is widely accepted that the level of digestive enzyme activity is a useful comparative indicator of food utilization rate, digestive capacity, and growth performance of the host (Suzer et al. 2008; Ueberschär 1995). Liu et al. (2017) also reported that 4-week *B. subtilis* HAINUP40 diet supplementation significantly increased protease and amylase activities of tilapia. This is because *B. subtilis* HAINUP40 could secrete exoenzymes; the improvement of indigestive tract enzyme activities may be partially due to enzymes synthesized by the bacteria. However, the proportion of enzymes contributed by bacteria cannot be assessed since the probiotic may also stimulate the production of endogenous enzymes in the fish (Dawood et al. 2016; Suzer et al. 2008; Wu et al. 2012; Ziaei-Nejad et al. 2006). In the same trend, supplementation of *B. subtilis* and *B. licheniformis* or *B. subtilis* and *Bacillus licheniformis* (BS) combined with traditional Chinese medicine (TCM) significantly enhanced weight gain and specific growth rate of Nile tilapia and Mozambique tilapia (Abarike et al. 2018b; Abarike et al. 2018a; Gobi et al. 2018). It is known that an increase in the body weight gain in fish fed with probiotic supplemented diets, could contribute to the increase in digestive enzyme activity, increase in

Table 1.1 Weight gain (WG), specific growth rate (SGR), food conversion efficiency (FCE), food conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), digestive enzyme, and disease resistance of fish and shellfish fed *Bacillus* probiotics. → no change, ↑ increase, ↓ decrease

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus licheniformis	Commercial probiotic	0%, 0.02%, 0.04%, 0.06%, 0.08% and 0.1% containing live germ 2×10^{10} CFU/g) 10 weeks	Juvenile Nile tilapia (*O. niloticus*) 3.83 ± 0.03 g	WG, FBW, SGR, and SR ↑ FCR → Villi length → Muscular layer thickness of anterior intestinal → Resistance against to *S. iniae* ↑	Han et al. (2015)
Bacillus subtilis (combined *with S. cerevisiae* and *A. oryzae)*	Commercial probiotic	0; 5 g kg^{-1} probiotic mixture (*B. subtilis* 1.5×10^9, *S. cerevisiae* 10^9 and *A. oryzae* 2×10^9); and 10 g kg^{-1} probiotic mixture (*B. subtilis* 3.0×10^9, *S. cerevisiae* 2.0×10^9 and *A. oryzae* 4.0×10^9) (CFU g^{-1}) 6 weeks	Juvenile Nile tilapia (*O. niloticus*) 25 ± 0.05 g	Growth rates → Resistance against to *A. hydrophila* and *S. iniae* ↑	Iwashita et al. (2015)
Bacillus amyloliquefaciens	Commercial probiotic	0; 1×10^6; 5×10^6 and 1×10^7 CFU g^{-1} 90 days	Nile tilapia (*O. niloticus*) 35 ± 5 g	Growth performance → Proximal composition → Blood glucose and hemoglobin ↓ Villi height and number of goblet cells ↑	Silva et al. (2015)

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Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus NP5	Commercial probiotic	10^{10} CFU g^{-1} in feed with dose of 0.5, 1 and 2% 28 days	Nile tilapia (*O. niloticus*) 6.38 ± 0.05 g	Growth performance ↑	Utami and Suprayudi (2015)
Bacillus subtilis (Aqua NZ and AP193)	Commercial probiotic	4.2 × 10^7 CFU g^{-1} of feed 8 weeks	Nile tilapia (*O. niloticus*) 7.47 ± 0.11 g	WG and FCR → Thermal growth coefficient → Resistance against *A. hydrophila* ↑	Addo et al. (2017a)
Bacillus subtilis strains SB3086, SB3295, SB3615, and AP193	Commercial probiotic	4 × 10^7 CFU/g of feed in 21 days	Nile tilapia (*O. niloticus*) 16.5 ± 0.2 g	Growth performance → Resistance against *Streptococcus agalactiae* ↑	Addo et al. (2017b)
Bacillus subtilis HAINUP40	Isolated from the aquatic environment	10^8 CFU/g 8 weeks	Nile tilapia (*O. niloticus*) 95 ± 8 g	FW, WG, and SGR ↑ FCR ↓ Protease and amylase activity ↑ Total antioxidant capacity (T-AOC) ↑ Serum superoxide dismutase (SOD) ↑ Resistance against *S. agalactiae* ↑	Liu et al. (2017)
B. subtilis and *B. licheniformis*	Commercial probiotic	0, 3, 5, 7 and 10 g kg^{-1} 4 weeks	Nile tilapia (*O. niloticus*) 53.01 ± 1.0 g	WG and SGR ↑ FCR ↓ Resistance against *S. agalactiae* ↑	Abarike et al. (2018a)

Bacillus subtilis and *Bacillus licheniformis* (BS) combined with traditional Chinese medicine (TCM)	Commercial probiotic	0; TCM at 3 and BS at 7 (g/kg); TCM at 5 and BS at 5 (g/kg); TCM at 7 and BS at 3 (g/kg). 4 weeks	Nile tilapia (*O. niloticus*) 57 ± 2 g	WG and SGR ↑ FCR ↓ Resistance against *S. agalactiae* ↑	Abarike et al. (2018a)
Bacillus licheniformis Dahb1	Commercial probiotic	0, 10^5 and 10^7 CFU/g 4 weeks	Mozambique tilapia (*Oreochromis mossambicus*)	FW and SGR ↑ FCR ↓ Resistance against *A. hydrophila* ↑	Gobi et al. (2018)
Bacillus licheniformis and *B. subtilis*	Commercial probiotic	1×10^6 CFU mL^{-1} was feed for *Artemia urmiana* nauplii and *Brachionus plicatilis* 8 h	Pacific white shrimp larvae (*Litopenaeus vannamei*)	Growth performance ↑ Survival rate ↑	Jamali et al. (2015)
Bacillus licheniformis and *Lactobacillus rhamnosus*	Commercial probiotic	*B. licheniformis* 10^9 CFU/kg, *L. rhamnosus* 8×10^8 CFU/kg 120 days	Pacific white shrimp (*L. vannamei*) PL14	WG, SGR, and WG ↑	Swapna et al. (2015)
Bacillus spp.	Isolated from pustulose ark	1×10^6, 2×10^6, 4×10^6, and 6×10^6 CFU g $feed^{-1}$ 32 days	Pacific white shrimp (*L. vannamei*) 1 ± 0.1 g	Growth performance ↑	Sánchez-Ortiz et al. (2016)
Bacillus coagulans ATCC 7050	Commercial probiotic	(0 (BO), 1×10^6 (BC1), 1×10^7 (BC2) and 1×10^8 (BC3) CFU g^{-1} feed) 56 days	Pacific white shrimp larvae (*L. vannamei*) 0.57 ± 0.001	FW, WG, and SGR ↑ FCR ↓ Condition factor ↑ Lipase, amylase, and trypsin ↑ Villus height ↑ Villus width ↑ Muscle thickness ↑	Amoah et al. (2019)

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Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus subtilis and *Bacillus licheniformis*	Commercial probiotic	1×10^4 and 1×10^8 CFU/g 60 days	Pacific white shrimp (*Litopenaeus vannamei*)	WG, total length, SGR, and SR ↑ Dry matter and crude protein ↑	Sadat Hoseini Madani et al. (2018)
Bacillus spp.	Isolate from shrimp gut	Added to water: 1×10^2, 1×10^3, 1×10^4 and 1×10^5 CFU/mL			Kewcharoen and Srisapoome (2019)
B. licheniformis	Commercial probiotic	Control diet (CON), 0.2% MOS (MOS), 0.1% *B. licheniformis* (BL), 0.2% MOS plus 0.1% BL (SYN) 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*)	WG, SGR, PER ↑ Villus number (VN) ↑ Villus height (VH) ↑ Thicker submucosa ↑ Propionic acid content ↑ Resistance against ammonia ↑	Chen et al. (2020a)
B. licheniformis		Basal diet (Control); 0.5% hydrolyzed yeast (HY); 0.1% *B. licheniformis* (BL) and 0.5% hydrolyzed yeast +0.1% *B. licheniformis* (SYN) 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*	Growth and body composition → PER ↑ Intestinal villus height ↑ Villus number (VN) ↑ Villus height (VH) ↑	Chen et al. (2020b)
Bacillus cereus and *Pediococcus acidilactici*	Commercial probiotic	*P. acidilactici* (106 CFU/mL) and *B. cereus* (106 CFU/mL) to the water pond 110 days	Pacific white shrimp (*Litopenaeus vannamei*) 0.002 ± 0.001	WG and SR ↑	Khademzade et al. (2020)

Bacillus subtilis WB60, Pediococcus pentosaceus, and *Lactococcus lactis*	Commercial probiotic	*B. subtilis* at 10^7 CFU/g diet, *B. subtilis*, *P. pentosaceus*, and *L. lactis* at 10^8 CFU/g diet, and oxytetracycline at 4 g/kg 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*) 1.41 ± 0.05	Growth performance ↑ Resistance against *Vibrio parahaemolyticus* ↑	Won et al. (2020a, 2020b)
Bacillus subtilis WB60 and *Lactobacillus plantarum* KCTC3928	Commercial probiotic	0; *B. subtilis* at 10^6, 10^7, 10^8 and *L. plantarum* at 10^6, 10^7, 10^8 CFU/g diet	Japanese eel (*Anguilla japonica*) 8.29 ± 0.06 g	WG, FE, and PER ↑ Resistance against *V. anguillarum* ↑	Lee et al. (2017)
Bacillus subtilis WB60 and mannanoligosaccharide (MOS)	Commercial probiotic	BS: 0.0, 0.5, and 1.0 × 107 CFU/g diet and MOS: 0 and 5 g/kg diet 8 weeks	Japanese eel (*Anguilla japonica*) 9.00 ± 0.11 g	WG, FW, SGR and PER ↑ Resistance against *Vibrio anguillarum* ↑	Lee et al. (2018)
Bacillus subtilis or *licheniformis*) and (mannan or fructo oligosaccharide)		0, Probiotics (1.0 × 10^8 CFU/g diet) and prebiotics (5 g/kg diet) 12 weeks	Japanese eel (*Anguilla japonica*) 12.8 ± 0.47	WG and SGR ↑ Intestinal villi length ↑ Resistance against *A. hydrophila* ↑	Park et al. (2020)
B. megaterium PTB 1.4	Commercial probiotic	0 and 1% 30 days	Catfish (*Clarias* sp.) 11.41 ± 0.23 g	Growth performance ↑ Protease and amylase enzymes ↑ Total amount of probiotic bacteria ↑	Afrilasari and Meryandini (2016)
B. subtilis, *B. amyloliquefaciens,* *B. cereus and a commercial* *B. amyloliquefaciens*	Isolated from the intestine of African catfish	10^{10} CFU/ml 60 days	African catfish (*Clarias gariepinus*) 75.23 ± 1.6	BW, WG, and SGR ↑ FCR ↓ Hemogram blood parameters ↑ Serum antioxidant and digestive enzymes ↑ Resistance against *Aeromonas sobria* ↑	Reda et al. (2018)

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Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus cereus EN25	Isolated from mud of sea cucumber culturing water bodies.	0, 10^5, 10^7 and 10^9 CFU/g 30 days	Juvenile sea cucumber (*Apostichopus japonicus*) 0.375 ± 0.024 g	Growth performance → Resistance against *V. splendidus* ↑	Zhao et al. (2016)
Bacillus baekryungensis MS1	Isolated from a sea cucumber pond in winter	0 and 10^7 CFU/ml 60 days	Sea cucumber (*Apostichopus japonicus*) 4.17 g ± 0.22 g	Growth performance ↑ Resistance to *Vibrio splendidus* ↑	Liu et al. (2020)
Bacillus subtilis and *Saccharomyces cerevisiae*	Commercial probiotic	*Bacillus subtilis* 10^9 UFC/g and *Saccharomyces cerevisiae* 10^9 UFC/g 90 days	Tambaqui (*Colossoma macropomum*) 2.13 ± 0.75 g	Growth performance → Body composition → Hematological parameters ↑ Resistance against *S. agalactiae* ↑	da Paixão et al. (2017)
Bacillus cereus	Commercial probiotic	0, 4.2 × 10^4, 3.9 × 10^6 and 3.3 × 10^8 CFU/g 120 days	Tambaqui (*Colossoma macropomum*) 0.94 ± 0.02 g	Weight and length gains ↑ Neutrophils and thrombocyte count ↑ Resistance against *Aeromonas hydrophila* ↑	Dias et al. (2018)
B. licheniformis and *B. subtilis*	Commercial probiotic	1.6 × 10^9 CFU/g dry pellet in 60 days	Kutum (*Rutilus frisii*) 0.4 ± 0.1 g	FW, WG, and SGR ↑ Red blood cells ↑ White blood cells ↑ Neutrophils ↑ Lymphocytes ↑ Mean cell volume ↑ Mean cell hemoglobin ↑ Mean cell hemoglobin concentration ↑	Azarin et al. (2015)

Bacillus sp. PP9	Isolated from mrigal gut	2×10^4, 2×10^5 and 2×10^6 CFU 60 days	Mrigal (*Cirrhinus mrigala*) 2.5 ± 0.20	Growth performance ↑ Maximum RNA DNA ratio ↑ FCR ↓ Intestinal protease and α-amylase activity ↑ Hepatic glutamic oxaloacetic transaminase ↑ Glutamate pyruvate transaminase levels ↑	Bandyopadhyay et al. (2015)
B. subtilis and *B. circulans*	Commercial probiotic	1×10^4, 2×10^4, 3×10^4, and 4×10^4 CFU/g) 30 days	Three spot gourami (*Trichopodus trichopterus*)	Larval growth → Larval resistance against the challenge ↑	Jafariyan et al. (2015)
Bacillus coagulant	Commercial probiotic	0, 10^5, 10^7 and 10^9 cfu g^{-1} 60 days	Freshwater prawn (*Macrobrachium rosenbergii*) 2.4 ± 0.35 g	Growth performance ↑ Feed utilization ↑ Protease, amylase, and lipase digestive enzymes ↑	Gupta et al. (2016)
Virgibacillus proomii and *Bacillus mojavensis*		5.8×10^4, 9.6×10^4, and 9.8×10^4 CFU/ml 60 days	Sea bass (*Dicentrarchus labrax*) larvae	Length, weight, and the survival rate ↑ FCR ↓ Phosphatase alkaline and amylase activities ↑	Hamza et al. (2016)
Bacillus spp.	Commercial probiotic	The live rotifers were enriched with: Algamac 3050; Algamac 3050 and a commercial mix of *Bacillus* spp. 0.5 g L^{-1}; additional probiotics in water (5 g m^{-3})	Florida pompano (*Trachinotus carolinus*) Larvae	Growth performance ↑ Survival rate → Trypsin-specific activity ↑ Alkaline phosphatase activity ↑	Hauville et al. (2016)

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Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus pumilus SE5		0 and 1.0 × 10^8 CFU g^{-1} 60 days	Grouper (*Epinephelus coioides*) 14.6 ± 0.2 g	FW, WG, and SGR ↑ FCR ↓	Yan et al. (2016)
Bacillus mycoides (BS) and organic selenium (OS)		BS 10^8 CFU g^{-1}, OS 0.2 g kg^{-1} and combination BS, OS	Marron (*Cherax cainii*) 10.83 ± 0.28 g	Growth performance ↑ The glutathione peroxidase ↑ Total hemocyte counts ↑ Intestinal bacterial population ↑	Ambas et al. (2017)
Bacillus amyloliquefaciens-JFP2		0 and 1.4 × 10^6 (CFU/g) of feed 90 days	Rock Bream (*Oplegnathus fasciatus*) 25.4 ± 0.13 g	BW, WG, and SGR ↑ FCR ↓ Serum protein and glucose level ↑ Resistance against *Streptococcus iniae* ↑	Kim et al. (2017)
B. siamensis B44v	Isolated from Thai pickled vegetables (Phak-dong)	10^7 CFU/g feed	Hybrid catfish (*C. macrocephalus* × *C. gariepinus*)	Protease and cellulase enzymes ↑ Gastrointestinal conditions ↑ Improve growth ↑ Resistance against to *A. hydrophila* and *S. iniae* ↑	Meidong et al. (2017)
Bacillus aerophilus KADR3	Commercial probiotic	0, 10^7, 10^8 and 10^9 CFU g^{-1} 6 weeks	Rohita labeo (*Labeo rohita*) 35–40 g	Serum lysozyme activity ↑ Phagocytic activity ↑ Serum total protein, Respiratory burst activity ↑	Ramesh et al. (2015)

				Serum IgM levels ↑ Superoxide dismutase activity ↑ Alternative complement pathway activity ↑ Resistance against *A. hydrophila* ↑	
Bacillus subtilis and *Bacillus cereus toyoi*	Commercial probiotic	0, 6 × 10^3 and 1.5 × 10^6 CFU g^{-1} of diet. 9 and 20 weeks	Rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) 15.6 g	Growth performance → Body composition → Intestinal *lamina propria* ↑ Submucosa ↑	Ramos et al. (2017)
B. amyloliquefaciens 54A and B. *pumilus* 47B	Isolated from gut of striped catfish	1 × 10^8, 3 × 10^8, and 5 × 10^8 CFU g^{-1} feed 90 days	Striped catfish (*Pangasianodon hypophthalmus*)	WG ↑ SGR and FCR → Resistance against to *E. ictaluri* ↑	Truong Thy et al. (2017)
Bacillus sp. DDKRC1	Isolated from the gut of Asian seabass (*Lates calcarifer*)	0, 2.94 × 10^7 CFU/100 g feed and diet fermented with *Bacillus* sp. DDKRC1 42 days	Tiger shrimp (*Penaeus monodon*) 2.73 ± 0.01	PER ↑ FCR ↓ Dry matter and cellulose digestibility ↑ Hemicellulose and lipid digestibility ↑ Cellulase, amylase, and protease activities ↑	De et al. (2018)
Bacillus amyloliquefaciens (GB) and Yarrowia lipolytica lipase 2 (YLL2)	Commercial probiotic	0, 5.0 g/kg GB-9, 4.0 g/kg YLL2, and 5.0 g/kg GB-9 + 4.0 g/kg YLL2 12 weeks	Hybrid sturgeon (*Acipenser schrenckii* ♂and *Acipenser baerii* ♀) 5.0 g	Final weight ↑ Docosahexaenoic acid (DHA) ↑ Eicosapentaenoic acid (EPA) concentration ↑	Fei et al. (2018)

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Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus licheniformis	Commercial probiotic	10^3, 10^5, and 10^7 CFU/mL 8 weeks	Abalone (*Haliotis discus* hannai Ino.) 4.17 ± 0.32 g	SGR and FI ↑ FCR ↓ Resistance to *V. parahaemolyticus* ↑	Gao et al. (2018)
B. subtilis E20	Commercial probiotic	0, 10^8, 10^9, and 10^{10} CFU kg^{-1} 56 days	Parrotfish (*Oplegnathus fasciatus*)	Growth performance ↑ Resistance against *V. alginolyticus* ↑	Liu et al. (2018)
Bacillus aerius B81e	Isolated from healthy hybrid catfish	0 and 10^7 CFU g^{-1} feed 60 days	Basa fish (*Pangasius bocourti*) 69 g	WG and SGR ↑ FCR ↓ Resistance against *A. hydrophila* ↑	Meidong et al. (2018)
Bacillus subtilis	Commercial probiotic	0, 1×10^4, 1×10^6, 1×10^8 and 1×10^{10} CFU kg^{-1} diet 60 days	Red sea bream (*Pagrus major*) 3.99 ± 0.01	FW, WG, and SGR ↑ Feed utilization (FI, FCE, PER and PG) ↑ Amylase, protease, and lipase enzymes ↑	Zaineldin et al. (2018)
BetaPlus® (*B. subtilis* (DSM 5750) *B. licheniformis* (DSM 5749)) and Isomaltooligosaccharides	Commercial probiotic	0 and 2 g kg^{-1} IMOS + 1 g kg^{-1} BetaPlus® in 7 weeks	Caspian Brown Trout (*Salmo trutta caspius*) 9 g	SR, BW, FW, and SGR ↑ FCR ↓ White blood cells ↑ Monocytes, neutrophils, and hematocrit ↑ Mean corpuscular volume and lymphocytes ↑ Serum triglycerides ↑ Cholesterol, total protein, and albumin ↑ Albumin/globulin ratio ↑	Aftabgard et al. (2019)

Bacillus subtilis and β-glucan	Commercial probiotic	1 g kg^{-1} β-glucan and 1 × 10^9 CFU kg^{-1} *B. subtilis* 70 days	Pengze crucian carp (*Carassius auratus* var. Pengze) 12.89 ± 0.04 g	Growth performance → Textures of muscle ↑ Cholesterol activity ↑ High-density lipoprotein ↑ Low-density lipoprotein ↑ Acid phosphatase activity ↑ Alkaline phosphatase activity ↑ Catalase activity ↑ Fold height and microvillus height ↑ Amylase, lipase, and trypsase activities ↑	Cao et al. (2019)
B. licheniformis and *B. amyloliquefaciens*	Commercial probiotic	(1) probiotics supplemented to the water and live feed, (2) probiotics supplemented to the water only, and (3) no probiotic controls with 1 × 10^{10} CFU g^{-1} in	Larval common snook (*Centropomus undecimalis*)	Growth performance ↑ Innate enzyme activities ↑ Inhibition of opportunistic bacteria ↑ Water quality parameters ↑	Tarnecki et al. (2019)
Bacillus subtilis	Isolated from the shrimp gut	2 × 10^2, 4 × 10^4, 6 × 10^6, 8 × 10^8 and 10 × 10^{10} CFU/100 g of feed 40 days	Indian prawn (*Penaeus indicus*) 16.8 ± 0.11 g	Bacterial growth ↑ Bacteriocin production ↑	Ock Kim et al. (2020)

(continued)

Table 1.1 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus licheniformis	Isolated from grass carp	1 × 10^5 cfu/g and 1 × 10^6 cfu/g 56 day	Grass carp (*Ctenopharyngodon idella*) 16.5 g	WG and SGR ↑ Resistance against *A. hydrophila* ↑	Qin et al. (2020)

appetite, increase in the production of vitamin, breakdown of indigestible components, as well as possible improvement of intestine morphology (Irianto and Austin 2002). In addition, *Bacillus* spp. could secret several digestive enzymes like protease, amylase, and lipase (Cai et al. 2019; Caulier et al. 2019).

1.3.1.2 Shrimps

Shrimp is a commercially important aquatic species with high economic value and good flavor, which has been widely farmed in the world, particularly in some Asia countries (Chen et al. 2020a). However, the shrimp industry has suffered severe economic losses because of the frequent outbreaks of diseases such as early mortality syndrome (EMS) and white spot syndrome virus (WSSV) (Alavandi et al. 2019; Castex et al. 2009; Chang et al. 2012). Chemotherapeutant and antibiotics are usually applied to settle this problem. Unfortunately, prolonged use of chemotherapeutant and antibiotics could lead to severe outcomes such as resistant bacteria, drug residues, and toxins, which pose a substantial threat to human beings and environment (Dash et al. 2015). Therefore, to seek an alternative way to solve this threat has caused increasing concern (Huynh et al. 2018). Probiotics have been widely applied in shrimp aquaculture. Jamali et al. (2015) revealed that dietary enrichment with *B. licheniformis* and *B. subtilis* significantly enhanced growth performance and survival rate of Pacific white shrimp, *Litopenaeus vannamei*. Elevation of growth performance has been demonstrated as the *Bacillus* could colonize shrimp digestive tract. In *P. monodon*, *Bacillus*, when used as a probiotic, was able to colonize both the culture water and the shrimp digestive tract; the *Bacillus* also was able to replace *Vibrio* spp. in the gut of the shrimp, thereby increasing shrimp survival (Rengpipat et al. 1998), via out-competing other bacteria for nutrients and space by producing antibiotics (Moriarty 1998; Verschuere et al. 2000). Similarly, significant improvement in growth performance of *L. vannamei supplemented with Bacillus* spp. *has been reported in previous studies* (Sadat Hoseini Madani et al. 2018; Sánchez-Ortiz et al. 2016; Swapna et al. 2015). Also, Amoah et al. (2019) indicated that dietary inclusion of *B. coagulans* significantly improved growth performance and feed utilization of Pacific white shrimp. The nutritive values as reported by Vijayavel and Balasubramanian (2006) is highly dependent on their biochemical constituents such as crude protein, crude lipid, ash content, and moisture, which also is noted to be an indication of improved meat quality. In addition, higher inclusion levels of probiotic BC at 1×10^8 CFU g^{-1} feed in diets could modulate gut microflora of *L. vannamei* (Amoah et al. 2019), which play an ardent role in the digestive enzyme activities and the intestinal health. It has been well documented that digestive enzymes are known to break down food and absorb nutrients (Gobi et al. 2018). The digestive enzymes including amylase, lipase, and trypsin (Rawlings and Barrett 1994; Svendsen 2000) in this study significantly increased in the treated group compared to the untreated. Similar results of improved digestive enzyme activities in *Litopenaeus vannamei* (Zokaeifar et al. 2012) and *Fenneropenaeus indicus* (Ziaei-Nejad et al. 2006) have been established. Verschuere et al. (2000) in their work also noted that, *Bacillus* genus secrets a wide range of exoenzymes which aid in the nutritional enhancement of the host. More recently, significant increase in growth performance, villus number, villus height, thicker submucosa, and propionic acid content has been reported in *L. vannamei* fed different *Bacillus* species (*B. licheniformis*, *B. cereus*, and *B. subtilis*) singularly or combined with other probiotics (*Pediococcus acidilactici*, *P. pentosaceus,* and *Lactococcus lactis*) (Chen et al. 2020a; Chen et al. 2020b; Khademzade et al. 2020; Won et al. 2020a).

Dietary supplementation of *Bacillus coagulans* on growth and feed utilization of freshwater prawn *Macrobrachium rosenbergii* showed that growth performance and feed utilization were found to be significantly higher ($P < 0.05$) in prawn fed 109 cfu g^{-1} diet. In addition, the specific activities of protease, amylase, and lipase digestive enzymes were significantly higher ($P < 0.05$) for 109 cfu g^{-1} diet (Gupta et al. 2016).

In Marron (*Cherax cainii*), Ambas et al. (2017) found that synbiotic use of *B. mycoides* and organic selenium (OS) significantly improved some immune parameters of marron, particularly the glutathione peroxidase, and to some extent total hemocyte counts. However, the synbiotic feed did not synergistically improve marron growth; in fact, the use of *B. mycoides*-supplemented diet alone demonstrated significantly higher growth in marron compared with the growth of marron fed on other test diets. A study conducted by Ock Kim et al. (2020), it was indicated that strain *Bacillus subtilis* isolated from the gut of *Penaeus indicus* and added at 2×10^2 CFU 100 g^{-1} as probiotics in feed, resulted in weight gain of the juvenile shrimp (16.8 ± 0.11 g) after 40 days. The weight gain was 16.8 ± 0.11 CFU 100 g^{-1} at 10×10^2 CFU 100 g^{-1} probiotic concentration.

1.3.1.3 Catfish

To the best of our knowledge, there were few studies regarding the use of *Bacillus* spp. on this fish. Afrilasari and Meryandini (2016) reported that *Bacillus megaterium* PTB 1.4 increased the activity of digestive enzymes and the growth of catfish. It is known that isolate PTB 1.4 is *B. megaterium,* where *Bacillus* spp. group is known to have ability to produce extracellular enzymes (Moriarty 1998). Probiotic bacteria are capable of producing digestive enzymes that help fish use feed nutrients and digest (Bairagi et al. 2002). Generally, endogenous enzyme can be produced by fish, but the presence of probiotics can improve digestive enzyme. Probiotics improve digestive enzyme activity by stimulating the synthesis of endogenous enzyme in the digestive tract (Mohapatra et al. 2012). Similarly, combination of *B. subtilis*, *B. amyloliquefaciens*, *B. cereus,* and a commercial *B. amyloliquefaciens* significantly improved growth performance of *C. gariepinus* (Reda et al. 2018). This improvement could be attributed to the production of amylase and protease by the same strain (Selim et al. 2019). In addition, *Bacillus* sp. are capable to detoxify the harmful substance in feed, produce essential vitamins such as vitamin B12 and biotin, and increase the intestinal villus heights (Ramirez and Dixon 2003; Reda and Selim 2015; Sugita et al. 1992).

In hybrid catfish (*C. macrocephalus* × *C. gariepinus*), Meidong et al. (2017) indicated that *Bacillus siamensis* strain B44v, selectively isolated from Thai pickled vegetables (Phak-dong), displayed a high potential as a probiotic in catfish culture.

Fish fed diet containing strain B44v (10^7 CFU g^{-1} feed) displayed not only no mortality but also growth improvement. The potential probiotic *B. siamensis* strain B44v could produce cellulase and protease, whereas the *Bacillus* sp. strain B51f produced protease and amylase enzymes. Ability to produce some hydrolytic enzymes is beneficial to the host. Enzymes increase the digestion of macromolecules in animal feed and improve feed intake by reducing digesta viscosity and increasing nutrient absorption in host animals (Ray et al. 2012).

For striped catfish, *Pangasianodon hypophthalmus*, the mixture of probiotics (*B. amyloliquefaciens* 54A and *B. pumilus* 47B) isolated from striped catfish at concentrations of 1×10^8, 3×10^8, and 5×10^8 CFU g^{-1} was added to the fish feed and conducted for 90 days. Truong Thy et al. (2017) reported that AWG (476.6 ± 7.81 g $fish^{-1}$) of fish fed probiotics at 5×10^8 CFU g^{-1} was significantly higher than the control (390 ± 25.7 g $fish^{-1}$) after 90 days of feeding, but there was no significant ($P > 0.05$) effect of probiotics on FCR and SGR. However, in basa fish, *Pangasius bocourti* (Meidong et al. 2018) reported that the administration of strain B81e isolated from the fish's gut (1×10^7 CFU g^{-1}) for 60 days had significant effects ($p < 0.05$) on weight gain, specific growth rate, and feed utilization efficiency of *P. bocourti*. This growth improvement might be related to the capability of the putative probiotics in producing extracellular protease and lipase within fish gut and thus exert beneficial effects to the digestive processes of the host fish as bacterial enzymes can help degrade the proteinaceous and lipid substrates (Ramesh et al. 2015; Ray et al. 2012). The significant reduction in FCR indicated that the fish utilized dietary nutrients more efficiently when feed was supplemented with strain B81e.

1.3.1.4 Japanese eel (*Anguilla japonica*)

Bacillus spp. supplementations have been recently applied in Japanese eel. Lee et al. (2017) indicated that dietary supplementation of *Bacillus subtilis* WB60 at 10^8 CFU g^{-1} in diet of Japanese eel (*Anguilla japonica*) resulted in better weight gain, feed efficiency, and protein efficiency ratio compared to the control and *Lactobacillus plantarum* diets. Similar results were observed in Japanese eel fed *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS), as well as (*Bacillus subtilis* or licheniformis) and (mannan or fructooligosaccharide) (Lee et al. 2018; Park et al. 2020). There is growing evidence that gastrointestinal bacteria facilitate the decomposition of nutrients in the host organism and provide physiologically active materials, such as enzymes, amino acids, and vitamins (Cencic and Chingwaru 2010; Morowitz et al. 2011; Wang et al. 2020a). These materials can positively influence the digestive tract and improve feed digestion and utilization (Bairagi et al. 2004; Dawood et al. 2019; Ramirez and Dixon 2003; Wang et al. 2020b).

1.3.1.5 Sea Cucumber (*Apostichopus japonicus*)

Supplementation of *Bacillus cereus* EN25 at 0 (control), 10^5, 10^7, and 10^9 CFU g^{-1} for 30 days showed no significant effects on growth of sea cucumbers *A. japonicus* (Zhao et al. 2016). Growth performance of sea cucumbers was one of the important indices to evaluate the effects of potential *Bacillus* spp. on culturing of sea cucumbers. Previous studies had proved that dietary *Bacillus* spp., such as indigenous *B. subtilis* T13 (Zhao et al. 2012), indigenous *B. cereus* (Yang et al. 2015), and commercial *B. subtilis* (Zhang et al. 2010), could improve the growth performance of sea cucumbers at suitable doses. This difference could be attributed to the differences in *Bacillus* strains, sizes of sea cucumbers, sources of sea cucumbers, experimental period, and experimental conditions. The present study was conducted with the same source of sea cucumbers at the same experimental period and conditions with Zhao et al. (2012), except *Bacillus* strain and initial sizes of sea cucumbers. Recently, Liu et al. (2020) indicated that dietary supplementation of *B. baekryungensis* MS1 at 10^7 cfu g^{-1} for a total of 60 days significantly improved the growth performance of the sea cucumber cultured under low temperature. This is related to the mode of action of probiotics, including the production of digestive enzymes, the production of antibacterial substances, immune stimulation, and interference of quorum sensing, all of which depend on the long-term growth and reproduction of probiotics. Studies have also shown that probiotics work by managing community assembly of the water and gut microbiota (Selim and Reda 2015; Wang et al. 2017a).

1.3.1.6 Tambaqui (*Colossoma macropomum*)

Dietary inclusion of *Bacillus subtilis* (10^9 UFC g^{-1}) and *Saccharomyces cerevisiae* (10^9 UFC g^{-1}) showed that no differences were found for the growth parameters between the treatments with probiotics (da Paixão et al. 2017). Although probiotics are supposed to be beneficial, the literature mentions possible synergistic effects. The total replacement of indigenous populations with probiotics may not be desirable to improve growth performance (Merrifield et al. 2010). The control of the endogenous balance between pathogenic and beneficial bacteria is still the target of many studies. According to Merrifield et al. (2010), the lack of improvements regarding growth and feed use may be explained by the level of gastrointestinal colonization that could be too high and any possible synergistic effect with the normal gut microbiota was negated. Thus, it is expected that the beneficial effects of probiotics for tambaquis are not on its performance but on its health and welfare. However, in another study with tambaqui, Dias et al. (2018) indicated that the use of the autochthonous bacteria *B. cereus* improves the growth performance, productivity, hematological profile, and survival of tambaqui juveniles. This enhanced growth performance of fish supplemented with probiotics is probably due to an improvement in digestion as well as an increase in the synthesis and absorption of nutrients (Hoseinifar et al. 2017). Similar results were obtained by El-Haroun et al. (2006) reporting increased growth performance and feed efficiency in tilapia fed the probiotics *Bacillus licheniformis* and *Bacillus subtilis*. According to these authors, the added probiotics improved digestibility, dietary protein, and energy utilization. These positive effects can be attributed to the capacity of the probiotics to promote an increase in the gut absorbent surface area, and stimulate and/or produce several enzymes on the intestinal tract, which improve digestibility and nutrient retention, leading to higher growth rates (El-Haroun et al. 2006; Ibrahem 2015).

1.3.1.7 Carp Species

Dietary administration of BioPlus 2B, a probiotic containing *Bacillus licheniformis* and *B. subtilis*, and Ferroin solution indicate that the combination of probiotic and Ferroin solution represents an effective dietary supplement for improving carcass quality, growth performance, and hematological parameters in kutum fry (Azarin et al. 2015). In mrigal fingerlings, *Cirrhinus mrigala* (avg.wt. 2.5 ± 0.20 g) were fed with three different doses (2×10^4, 2×10^5, and 2×10^6 CFU) of *Bacillus* sp. PP9 admixed with 100 g feed for a period of 60 days. It was found that the feed with Bacillus concentration of 2×10^4 CFU exhibited significantly higher growth and lower food conversion ratio compared to the control and other supplemented diets (Bandyopadhyay et al. 2015). More recently, Qin et al. (2020) found that dietary inclusion of *B. licheniformis* at the low-dose 1×10^5 cfu g^{-1} and the high-dose (HD) group with 1×10^6 cfu g^{-1} led to significantly ($p < 0.05$) improved percent weight gain (PWG) and specific growth rate (SGR) parameters. The improvement of growth performance parameters such as PWG and SGR with increasing concentrations of supplemented *B. licheniformis* FA6 observed in this study is in agreement with Han et al. (2015) observed a significant increase in the growth performance of tilapia fed with *B. licheniformis*. The increase in the growth performance of grass carp may due to the secretion of digestive enzymes by *B. licheniformis*, which improves feed digestibility (Kuebutornye et al. 2019).

In Pengze crucian carp, *Carassius auratus*, dietary supplementation with prebiotics β-glucan (BG group) and probiotics *Bacillus subtilis* (BS group) resulted in better growth performance than other groups whereas feed efficiency was unaffected by dietary treatments. The textures of muscle in terms of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were higher in BG and BS groups than the control group. Supplementation of β-glucan and *B. subtilis* acted as a hypolipidemic in terms of decreasing the total cholesterol, high-density lipoprotein, and low-density lipoprotein, whereas increased the immune responses in serum measured by acid phosphatase, alkaline phosphatase, and catalase activities. Dietary supplementation of β-glucan and *B. subtilis* significantly improved the fold height and microvillus height in contrast to basal diet. Moreover, β-glucan could significantly increase digestive capacity observed in terms of an increase in amylase and trypsase activities, and *B. subtilis* significantly increased amylase and lipase activities in intestine (Cao et al. 2019).

1.3.1.8 Trout

A commercial probiotic (4.2×10^9 CFU g^{-1} of additive) was supplemented to the experimental diets at 0% (control), 0.03% (P_1; 6×10^3 CFU $g^{-1}$of diet), or 0.06% (P_2; 1.5×10^6 CFU g^{-1} of diet) and fed to brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) for 9 and 20 weeks, respectively. Rainbow trout showed significantly better growth performance than brown trout, regardless of the dietary treatment. No effect of dietary probiotic supplementation was detected on growth performance and body composition (Ramos et al. 2017). However, in Caspian Brown Trout (*Salmo trutta caspius*)Aftabgard et al. (2019) found that the combined effects of IMOS, a prebiotic, and BetaPlus®, a probiotic containing

B. subtilis and *B. licheniformis*, demonstrated a better performance of select growth indices, including BWI and FCR, than fish that were fed the control diet; these results were probably due to improved nutrition and digestive processes (Cerezuela et al. 2011).

1.3.1.9 Other Aquacultured Species

Two probiotics (*Virgibacillus proomii* and *Bacillus mojavensis*) were used to study their effects on the digestive enzyme activity, survival, and growth of sea bass, *Dicentrarchus labrax* at various ontogenetic stages in three separate experiments (Hamza et al. 2016). The results indicated that the two probiotics *V. proomii* and *B. mojavensis* were adequate for improved growth performance and survival and for healthy gut microenvironment of the host (Hamza et al. 2016).

In the study of Hauville et al. (2016) Florida pompano (*Trachinotus carolinus*) larvae were fed either live feed enriched with Algamac 3050 (Control), Algamac 3050, and probiotics (PB), or the previous diet combined with a daily addition of probiotics to the tank water (PB+). The results indicated that a mix of *Bacillus* sp. can promote growth through an early maturation of the digestive system during the early larval stages of pompano and snook.

In grouper *Epinephelus coioides* (Yan et al. 2016, juveniles (14.6 ± 0.2 g) were fed either a basal control diet (without probiotic) or the basal diet supplemented with 1.0×10^8 CFU g^{-1} live (T1) and heat-inactivated *B. pumilus* SE5 (T2). The results indicated that the heat-inactivated probiotic significantly improved the final weight, weight gain (WG), and specific growth rate (SGR) at day 60 and significantly decreased the feed conversion ratio (FCR) at day 30 and 60, while the viable probiotic significantly decreased the FCR at day 60 ($P < 0.05$). This suggested that live and heat-inactivated *B. pumilus* could promote the efficient utilization of dietary nutrients. Interestingly, significant increased growth was only observed in fish fed the heat-inactivated *B. pumilus* containing diet for 60 days, but not in fish fed the live *B. pumilus* containing diet. Likewise, Hoseinifar et al. (2011) observed that dietary supplementation of 20 g kg^{-1} inactive brewer's yeast *Saccharomyces cerevisiae var. ellipsoideus* significantly improved the growth performance in juvenile beluga sturgeon (*Huso huso*). In rock bream, *Oplegnathus fasciatus*, Kim et al. (2017), revealed that supplementation of *B. amyloliquefaciens* spores at a concentration of 1.4×10^6 colony-forming units per gram (CFU g^{-1}) of feed for 90 days resulted in significant improvements in body weight (BW), weight gain (WG), specific growth rate (SGR), and food conversion ratio (FCR) when compared with control group fish.

In hybrid sturgeon, *Acipenser schrenckii* ♂ and *Acipenser baerii* ♀, fish were fed with *Bacillus amyloliquefaciens* (GB-9) and *Yarrowia lipolytica* lipase2 (YLL2): Diet 1 (0-control), Diet 2 (5.0 g kg^{-1} GB-9), Diet 3 (4.0 g kg^{-1} YLL2), and Diet 4 (5.0 g kg^{-1} GB-9 + 4.0 g kg^{-1} YLL2), respectively (Fei et al. 2018). The results indicated that supplementations of GB-9 + YLL2 resulted in a significant increase in final weight, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) concentration, compared with that of control ($p < 0.05$). This might be because the DHA and EPA hydrolyzed by YYL2 improved the poor establishment of the GB-9 in the gastrointestinal tract of hybrid sturgeon and might have promoted the growth of GB-9 (Menni et al. 2017). Similarly, combination of *B. licheniformis* and *B. amyloliquefaciens* indicated up to 2.5 times higher survival with probiotic addition, as well as 20% higher survival 7 days following a transport event. These benefits could not be explained by faster larval growth. In fact, CONT larvae were significantly longer than probiotic-treated larvae, likely due to decreased competition for food in CONT tanks which exhibited significantly lower survival. The other differing morphometric in this study was oil globule volume which was lowest in CONT larvae, suggesting that CONT larvae were consuming their endogenous reserves more quickly than probiotic-treated larvae. Retention of oil globules allows for a longer transition time to exogenous feeding, and studies indicate larvae that retain their endogenous reserves longer demonstrate increased survival (Avila and Juario 1987; Berkeley et al. 2004). The probiotic may alter development of the digestive tract and thus the start of exogenous feeding, as has been demonstrated in previous studies involving *Bacillus* probiotics and common snook (Hauville et al. 2016).

Dietary supplementation of *B. subtilis* has been reported to improve the growth performance, feed utilization, amylase, protease, and lipase enzymes of parrotfish (*Oplegnathus fasciatus*) and red sea bream (*Pagrus major*) (Liu et al. 2018; Zaineldin et al. 2018). The observed improvement in growth performance might be ascribed to the enhanced intestinal digestive enzyme activity and beneficial intestinal microbiota (Dawood et al. 2014; Liu et al. 2009; Sun et al. 2010). *Bacillus* sp. can produce certain essential micronutrients to promote better growth and feed utilization of hosts (Sanders et al. 2003). Further, *Bacillus* species may participate in digestion processes to break down nutrients such as carbohydrates, proteins, and lipids by producing extracellular enzymes (Liu et al. 2009; Sun et al. 2010). In abalone, *Haliotis discus hannai*, Gao et al. (2018) indicated that the food containing 10^5 cfu mL^{-1} *Bacillus licheniformis* promoted food intake and growth of abalones. *Bacillus licheniformis* is an aerobic nonpathogenic bacterium that inhabits the intestinal microbial community in the form of spores, which can reduce intestinal pH, reduce ammonia concentration, and promote decomposition of starch and cellulose. Thus, it is generally considered to be a relatively stable probiotic (Hong et al. 2005; Vine et al. 2006).

1.3.2 Increase Disease Resistance

Probiotics have been proven as an effective tool for disease prevention in aquaculture (Hoseinifar et al. 2018). Probiotics can interact with or antagonize other enteric bacteria by resisting colonization or by directly inhibiting and reducing the incidence of opportunistic pathogens (Chiu et al. 2017). They can also improve host's health and well-being via physiological or immune modulation (Butt and Volkoff 2019). Probiotics can produce effective molecules that have bactericidal activity on intestinal pathogenic bacteria of the host, providing a barrier against the proliferation of opportunistic pathogens (Martínez Cruz et al. 2012; Seghouani et al. 2017). The functional molecules produced during the bactericidal activity are antibiotics, bacteriocins, siderophores, enzymes and/or hydrogen peroxide as well as the alteration of the intestinal pH due to the generation of organic acids (Verschuere et al. 2000). The inhibition of intestinal related diseases has been reported in several cultured species by probiotic incorporation in aquafeeds (Ringø et al. 2018; Serra et al. 2019; Wanka et al. 2018). Thus, it can be confirmed that the ability of aquatic animals to avoid the infectious diseases mainly depends on the immunomodulatory effect that happened due to the administration of beneficial bacterial cells.

1.3.2.1 Tilapias

Dietary inclusion of *B. licheniformis* at 0%, 0.02%, 0.04%, 0.06%, 0.08%, and 0.1% containing live germ 2×10^{10} CFU/g for 10 weeks significantly increased disease resistance of Nile tilapia, *Oreochromis niloticus* against *Streptococcus iniae* (Han et al. 2015). *Bacillus* strains supplementation in diet could increase disease resistance in fish through the stimulation of both the cellular and humoral immune function, such as phagocytic activity, lysozyme activity, and complement activity (Arena et al. 2006; Queiroz and Boyd 1998; Sookchaiyaporn et al. 2020; Zhou et al. 2010). It was reported that *Bacillus* bacteria are able to outcompete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics, and as usually lead to the enhanced immunity of fish (Cha et al. 2013). Similarly, dietary inclusion of *B. licheniformis* Dahb1 at 10^7 cfu g^{-1} could improve disease resistance of Mozambique tilapia (*Oreochromis mossambicus*) against *A. hydrophila* (Gobi et al. 2018). In terms of *Bacillus subtilis* HAINUP40, H. Liu et al. (2017) reported that dietary supplement of *B. subtilis* HAINUP40 at 10^8 cfu g^{-1} can effectively enhance disease resistance of Nile tilapia against *Streptococcus agalactiae*. In addition, combination of *B. subtilis* with *S. cerevisiae* and *A. oryzae*; *Bacillus subtilis* with Aqua NZ and AP193; *Bacillus subtilis* strains SB3086, SB3295, SB3615 with AP193; *B. subtilis* and *B. licheniformis*, and *Bacillus subtilis* and *Bacillus licheniformis* (BS) combined with traditional Chinese medicine (TCM) *A. hydrophila* and *S. iniae*. Higher intestinal *Bacillus* spp. counts can regulate the gut microbiota of fish, selectively stimulate other beneficial probiotic bacteria, and depress some potential harmful bacteria (Yang et al. 2012).

1.3.2.2 Shrimps

The efficiency of these isolates in controlling pathogens, which is a key factor in selecting appropriate bacteria as probiotics, was evaluated (Kesarcodi-Watson et al. 2008). Based on in vitro laboratory results, B4, B6, and B12 inhibited *V. parahaemolyticus*; however, only *B. subtilis* AQHPS001 (B12) showed the highest antagonistic property against VP_{AHPND} strains. However, among the VP_{AHPND} strains, there were different sizes of the inhibitory clear zone, and $VP_{AHPNDAQH3.2}$ was the only strain that resisted B12. This suggests that there are varieties of VP_{AHPND} and that each strain may employ different mechanisms in response to the target B12 (Kewcharoen and Srisapoome 2019). Previous reports found that *Bacillus* spp. could produce many kinds of bacteriocins, such as subtilin, subtilosin, coagulin, megacin, bacillin, bacillomycin, mycosubtilin, toximycin, and xanthobacidin, which could reduce pathogen colonization by directly inhibiting pathogens while having no resulting effects on the virulence resistance genes of pathogenic bacteria (Desriac et al. 2010; Hammami et al. 2012; Joseph et al. 2013). Zhao et al. (2015) also reported that *Bacillus* spp. could secrete quorum-quenching enzymes, which are expected to be quorum-sensing blockers to reduce disease infection. These results suggest that *B. subtilis* AQAHBS001 possesses more effective characteristics that are important for controlling the various harmful VP_{AHPND} strains than other candidates. For these reasons, it was further chosen to study its application on a laboratory scale. Similarly, dietary inclusion of *Bacillus subtilis* WB60, *Pediococcus pentosaceus*, and *Lactococcus lactis* at 10^8 CFU g^{-1} could improve disease resistance of whiteleg shrimp *Litopenaeus vannamei* against *Vibrio parahaemolyticus* (Won et al. 2020b). Generally, administration of probiotics in the shrimp diet was shown to decrease mortality rates compared to the CON diet (Balcázar et al. 2007; Sapcharoen and Rengpipat 2013; Zhang et al. 2009). Previous studies demonstrated that probiotic supplementation can be used for modulating fish health and disease resistance (Wang et al. 2018; Zuo et al. 2019). Indeed, probiotics can beneficially influence the disease resistance of fish to pathogen bacteria by producing antimicrobial substances and competing with pathogens for physical occupation of space (Lim et al. 2020). As a result, the enhanced survival and cumulative survival rates could be due to probiotic supplementation. Chen et al. (2020a) recently indicated that dietary MOS and/or *B. licheniformis* supplementation could positively increase ammonia resistance of *Litopenaeus vannamei*. According to Chen et al. (2012), immune parameters decrease after ammonia stress, yet these parameters recover faster when they were initially stimulated by a probiotic. Faster recovery of immune parameters might have contributed to the increased survival after ammonia stress for the *Rps. palustris* fed shrimp.

1.3.2.3 Catfish

Meidong et al. (2017) revealed that *Bacillus siamensis* strain B44v and *Bacillus* sp. strain B51f, derived from indigenous fermented foods, displayed strongly antagonistic activity against the bacterial fish pathogens, *A. hydrophila* and *S. agalactiae*. Both strains effectively inhibited Gram-positive and Gram-negative bacteria, indicating their broad spectrum as a useful antagonistic property as the two most striking bacterial fish pathogens in aquaculture in Thailand belong to the genera the *Aeromonas* and *Streptococcus* (Maisak et al. 2013). Besides fish pathogens, the bacteriocin-like substance from *B. siamensis* strain B44v inhibited several foodborne pathogens suggesting potential applications in human foods (Sivamaruthi et al. 2018). Likewise, Reda et al. (2018) showed that supplementation of three autochthonous *Bacillus strains* (*B. subtilis*, *B. amyloliquefaciens*, and *B. cereus*) and a commercial *B. amyloliquefaciens* at a dose of 1×1010 CFU kg^{-1} significantly increased disease resistance of African catfish against *Aeromonas sobria*. This may be returned to the ability of *Bacillus* spore to resist gastrointestinal conditions, survive and transit cross gastrointestinal tract, germinate and vegetate with heterologous antigen expression before being excreted (Duc et al. 2003). In striped catfish, Truong Thy et al. (2017) indicated that the mixed probiotics of *Bacillus* *amyloliquefaciens* 54A and *B. pumilus* 47B isolated from striped catfish (*Pangasianodon hypophthalmus*) intestine significantly enhanced disease resistance of the fish against *Edwardsiella ictaluri* and ammonia tolerance. Antimicrobial activity of probiotics has been demonstrated on many in vitro and in vivo studies in animals. The study of Corr et al. (2007) reported trial mice received protection from *Lactobacillus salivarius* against *Listeria monocytogenes* involved bacteriocin produced by *L. salivarius*UCC118. Additionally, antimicrobial activities of probiotics against pathogens include secretion of hydrogen peroxide (Pridmore et al. 2008), lactic acid (Fayol-Messaoudi et al. 2005), competitive exclusion (Lee et al. 2003), and stimulation of immune system (Ryan et al. 2009). The positive effect on barrier function of probiotics is to protect the host intestine by prevention of pathogen attachment to epithelial cells on gut surface (Mennigen et al. 2009). In basa fish, *Pangasius bocourti*, Meidong et al. (2018) found that *B. aerius* B81e has beneficial effects on growth performance, innate immunity, and disease resistance of *P. bocourti* against *Aeromonas hydrophila* and *Streptococcus agalactiae*. Bacterial co-aggregation has considerable significance in the host gut as co-aggregation ability of bacterial probiotics might interfere with the ability of pathogenic bacteria to infect the host and can prevent colonization of the pathogens (Spencer and Chesson 1994). In addition, *B. aerius* B81e has an absence of hemolysin and is susceptible to most of the common antibiotics tested which demonstrated that it is likely a nonpathogen and has an inability to transfer antibiotic-resistant genes to recipient bacteria in the host gut, thus preventing the development of antibiotic-resistant pathogens (Meidong et al. 2018).

1.3.2.4 Japanese eel (*Anguilla japonica*)

The combination of *Bacillus subtilis* WB60 and *Lactobacillus plantarum* KCTC3928 or *Bacillus subtilis* WB60 and mannanoligosaccharide (MOS) significantly improved disease resistance of Japanese eel against *V. anguillarum* (Lee et al. 2017, 2018). Similarly, Park et al. (2020) reported that dietary inclusion of *B. subtilis* with FOS (BSF) and *B. licheniformis* significantly increased disease resistance against *Aeromonas hydrophila*. Significant increase in disease resistance in these works may be attributable to the stimulation of cellular and humoral immune function.

1.3.3 Sea Cucumber (*Apostichopus japonicus*)

Zhao et al. (2016) indicated that the cumulative mortality after *V. splendidus* challenge decreased significantly in sea cucumbers fed with EN25 at 10^7 CFU g^{-1} ($P < 0.05$). The present study confirmed dietary *B. cereus* EN25 at 10^7 CFU g^{-1} could significantly improve disease resistance in juvenile *A. japonicus*. Recently, Liu et al. (2020) showed that *B. baekryungensis* MS1 significantly reduced the mortality of sea cucumbers infected with *Vibrio splendidus*. By regulating the expression of immune-related genes and signaling pathways, B. *baekryungensis* MS1 improved the immunity of sea cucumber in winter and effectively controlled the infection of pathogenic bacteria such as *V. splendidus*.

1.3.4 Tambaqui (*Colossoma macropomum*)

da Paixão et al. (2017) indicated that supplementation of two probiotics *Bacillus subtilis* and *Saccharomyces cerevisiae* at 10^9 UFC g^{-1} significantly increased disease resistance of tambaqui, *Colossoma macropomum*, against *Streptococcus agalactiae*. Similarly, Dias et al. (2018) reported that *B. cereus* (4.2×10^4, 3.9×10^6 and 3.3×10^8 CFU g^{-1}) supplemented as probiotics to *C. macropomum* for 120 days significantly increased disease resistance against *Aeromonas hydrophila*. The probiotic promoted a nonspecific response against bacterial infection, increasing fish survival after challenge with *A. hydrophila*.

1.3.5 Other Species

In rock bream, *Oplegnathus fasciatus*, Kim et al. (2017) demonstrated the benefit of incorporation of *B. amyloliquefaciens* as a feed supplement to improve the health status of *Oplegnathus fasciatus* challenged with *Streptococcus iniae*. The enhancement of the innate immune response with a *B. amyloliquefaciens* enriched probiotic diet and decreased mortality rate, thereby protecting the fish against *S. iniae*. Similarly, dietary inclusion of *B. subtilis* at 10^8 CFU kg^{-1} significantly increased disease resistance of parrotfish, *Oplegnathus fasciatus*, against *Vibrio alginolyticus* (Liu et al. 2018). The growth performance and health status improvement of aquatic animal might be involved with the gut microbiota change after probiotic administration. The previous study has also demonstrated the positive effects of *B. subtilis* E20 in terms of intestinal presence and subsequent health benefits for *L. vannamei* (Liu et al. 2009; Tseng et al. 2009) and *E. coioides* (Liu et al. 2010). In the same trend, dietary inclusion of *Bacillus licheniformis* significantly improved disease resistance of abalone, *Haliotis discus hannai Ino.,* against *V. parahaemolyticus* and grass carp, *Ctenopharyngodon idella,* against *A. hydrophila* (Gao et al. 2018; Qin et al. 2020).

1.4 Immune Effects of *Bacillus*

Enhancement of host immunity is one important benefit of probiotic diet supplementation (Kuebutornye et al. 2019). As stated by Verschuere et al. (2000), probiotics can modulate innate immunity through the modulation of humoral immune responses and expression of immune-related genes. Effects of Bacillus on immune response of different fish and shellfish are displayed in Table 1.2.

Table 1.2 Immune responses of fish and shellfish fed different *Bacillus* probiotics. → no change, ↑ increase, ↓ decrease

Species	Source	Doses and duration	Fish species	Parameters investigated	References
B. amyloliquefaciens	Commercial probiotic	0; 1 × 10^4 and 1 × 10^6 CFU/g 30 days	Nile tilapia (*O. niloticus*) 27.7 ± 0.22 g	Serum killing percentages ↑ Phagocytic activities ↑ Lysozyme activities ↑ Nitric oxide assay ↑ The percentage of serum killing ↑ Serum nitric oxide activity ↑ Serum lysozyme activity ↑	(Selim & Reda 2015)
Bacillus subtilis (combined with S. cerevisiae and *A. oryzae)*	Commercial probiotic	0; 5 g kg^{-1} probiotic mixture (*B. subtilis* 1.5 × 10^9, *S. cerevisiae* 10^9 and *A. oryzae* 2 × 10^9); and 10 g kg^{-1} probiotic mixture (*B. subtilis* 3.0 × 10^9, *S. cerevisiae* 2.0 × 10^9 and *A. oryzae* 4.0 × 10^9) (CFU g^{-1}) 6 weeks	Juvenile Nile tilapia (*O. niloticus*) 25 ± 0.05 g	Respiratory burst activity ↑ Erythrocyte fragility ↑ Levels of white blood cells ↑	(Iwashita et al. 2015)
Bacillus licheniformis	Commercial probiotic	0%, 0.02%, 0.04%, 0.06%, 0.08% and 0.1% containing live germ 2 × 10^{10} (CFU/g) 10 weeks	Juvenile Nile tilapia (*O. niloticus*) 3.83 ± 0.03 g	Lysozyme activity ↑ Content of complement C3 ↑	(Han et al. 2015)
B. subtilis and *B. licheniformis*	Commercial probiotic	0, 3, 5, 7 and 10 g kg^{-1} 4 weeks	Nile tilapia (*O. niloticus*) 53.01 ± 1.0 g	Lysozyme and protease activity ↑ Anti-protease activity ↑ Superoxide dismutase activity ↑	(Abarike et al. 2018a)

				Immunoglobulin M level ↑ Myeloperoxidase activity ↑ Expression of C-lysozyme ↑ Heat shock protein 70 ↑ β-defensin ↑ Transforming growth factor beta ↑ Small body size decapentaplegic homolog 3 ↑	
Bacillus subtilis and *Bacillus licheniformis* (BS) combined with traditional Chinese medicine (TCM)	Commercial probiotic	0; TCM at 3 and BS at 7 (g/kg); TCM at 5 and BS at 5 (g/kg); TCM at 7 and BS at 3 (g/kg) 4 weeks	Nile tilapia (*O. niloticus*) 57 ± 2 g	Lysozyme activity ↑ Superoxide dismutase activity ↑ Catalase, protease, and antiprotease ↑ Expression of C-lysozyme ↑ Heat shock protein 70 ↑ β-defensin ↑ Transforming growth factor beta ↑	(Abarike et al. 2018b)
Bacillus licheniformis Dahb1	Commercial probiotic	0, 10^5 and 10^7 CFU/g 4 weeks	Mozambique tilapia (*Oreochromis mossambicus*)	Alkaline phosphatase ↑ Myeloperoxidase ↑ Lysozyme ↑ Reactive oxygen species ↑ Reactive nitrogen species ↑ Superoxide dismutase ↑ Glutathione peroxidase ↑	(Gobi et al. 2018)
Bacillus subtilis HAINUP40	Isolated from the aquatic environment	10^8 CFU/g 8 weeks	Nile tilapia (*Oreochromis niloticus*) 95 ± 8 g	Respiratory burst activity ↑ Serum lysozyme activity ↑	(Liu et al. 2017)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus cereus	Commercial probiotic	Exp 1: 0, 1.0×10^4 and 1.0×10^5 CFU mL^{-1} in 42 days on tanks Exp 2: 0, 1.0×10^7 and 1.0×10^8 CFU g^{-1} 42 days on cages	Nile tilapia (*Oreochromis niloticus*) 20.20 ± 0.17 g	Serum lysozyme ↑ Peroxidase activity ↑ Alkaline phosphatase activity ↑ Total superoxide dismutase activity ↑ Autochthonous gut bacteria community ↑ Potentially beneficial bacteria ↑	(Wang et al. 2017b)
Bacillus pumilus AQAHBS01	Isolated from farmed fish	10^6, 10^7, 10^8 and 10^9 CFU/g 30 days	Nile tilapia (*Oreochromis niloticus*) 50 g	Phagocytic activity ↑ Superoxide anion levels ↑	(Srisapoome & Areechon 2017)
Bacillus subtilis strains SB3086, SB3295, SB3615, and AP193	Commercial probiotic	4×10^7 CFU/g of feed in 21 days	Nile tilapia (*Oreochromis niloticus*) 16.5 ± 0.2 g	Serum bactericidal activity ↑ Lysozyme activity ↑	(Addo et al. 2017b)
Bacillus subtilis (Aqua NZ and AP193)	Commercial probiotic	4.2×10^7 CFU g^{-1} of feed 8 weeks	Nile tilapia (*Oreochromis niloticus*) 59.5 ± 0.99 g	Lysozyme activity → Respiratory burst activity →	(Addo et al. 2017a)
*Bacillus cereus*NY5 and *Alcaligenes faecalis* Y311	Nile tilapia intestine	1.0×10^4 CFU mL^{-1} 3 months	Nile tilapia (*Oreochromis niloticus*) 5.20 ± 0.17 g	The total superoxide dismutase activities ↑ Alkaline phosphatase activities ↑	(Wang et al. 2020a, 2020b)

Bacillus licheniformis HGA8B		1×10^6 and 1×10^8 CFU g^{-1} 60 days	Pacific white shrimp (*Litopenaeus vannamei*) 0.57 ± 0.001 g	Lysozyme activity ↑ Phosphatase activity ↑ Superoxide dismutase (SOD) ↓ Total protein (TP) ↑ Albumin (ALB) in serum ↑ Glutathione peroxidase (GSH-Px) ↑	(Amoah et al. 2019)
Bacillus subtilis and *Bacillus licheniformis*	Commercial probiotic	1×10^4 and 1×10^8 CFU/g 60 days	Pacific white shrimp (*Litopenaeus vannamei*)	Lysozyme and hemocyte cell count ↑	(Sadat Hoseini Madani et al. 2018)
B. licheniformis	Commercial probiotic	Control diet (CON), 0.2% MOS (MOS), 0.1% *B. lincheniformis* (BL), 0.2% MOS plus 0.1% BL (SYN) 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*)	Expression levels of catalase ↑ Expression levels of glutothion peroxidase ↑ Expression of superoxide dismutase (SOD) ↑ Expression of penaeidin -3a (Pen-3a) ↑ Expression of heat shock protein (Hsp-70 ↑	(Chen et al. 2020a)
B. licheniformis		Basal diet (Control); 0.5% hydrolyzed yeast (HY); 0.1% *B. lincheniformis* (BL) and 0.5% hydrolyzed yeast + 0.1% *B. licheniformis* (SYN) 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*) 2.0 ± 0.01 g	GPX, SOD, and ACP ↑ MDA ↓ Expression of CAT, GPX, and SOD ↑ Expression of Pen-3a and PPO ↑ Pen-3a and SOD ↓	(Chen et al. 2020b)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus licheniformis MAt32, *B. subtilis* MAt43 and *B. subtilis* subsp. *subtilis* GAtB1	Isolated from pustulose ark *Anadara tuberculosa*	0, 1 × 10^6, 2 × 10^6, 4 × 10^6, and 6 × 10^6 CFU g^{-1} of feed. 32 days	Pacific white shrimp (*Litopenaeus vannamei*) 1 ± 0.1 g	Expression of proPO gene ↑ Expression of LvToll1 and SOD genes ↑ expression of the Hsp70 gene ↓ Expression of TGase gene →	(Sánchez-Ortiz et al. 2016)
Bacillus spp	Isolated from pustulose ark	1 × 10^6, 2 × 10^6, 4 × 10^6, and 6 × 10^6 CFU g feed^{-1} 32 days	Pacific white shrimp (*L. vannamei*) 1 ± 0.1 g	proPO gene ↑ LvToll1 gene ↑ SOD gene ↑ TGase gene →	(Sánchez-Ortiz *et al.* 2016)
Bacillus subtilis WB60, Pediococcus pentosaceus, and *Lactococcus lactis*	Commercial probiotic	*B. subtilis* at 10^7 CFU/g diet, *B. subtilis*, *P. pentosaceus*, and *L. lactis* at 10^8 CFU/g diet, and oxytetracycline at 4 g/kg 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*) 1.41 ± 0.05 g	Superoxide dismutase activity ↑ Lysozyme activity ↑ Immune-related gene expression ↑	(Won et al. 2020)
Bacillus cereus and *Pediococcus acidilactici*	Commercial probiotic	*P. acidilactici* (10^6 CFU/mL) and *B. cereus* (10^6 CFU/mL) to the water pond 110 days	Pacific white shrimp (*Litopenaeus vannamei*) 0.002 ± 0.001 g	Total hemocyte count ↑ Total protein ↑ Lysozyme activity ↑	(Khademzade et al. 2020)
Bacillus aryabhattai TBRC8450	Commercial probiotic	1 × 10^8 CFU/g diet 6 weeks	Pacific white shrimp (*Litopenaeus vannamei*) 0.9 ± 0.1 g	C-type lectin ↑ Penaeidin-3 ↑ Heat shock protein 60 ↑ Thioredoxin, and ferritin ↑ phenoloxidase activity ↑ Total antioxidant activity ↑ Total hemocyte count → Superoxide dismutase →	(Tepaamorndech et al. 2019)

Bacillus subtilis E20		10^9 cfu (kg diet)$^{-1}$ 8 weeks	Pacific white shrimp (*Litopenaeus vannamei*) 3.78 ± 0.21 g	Antioxidant enzymes gene ↑ Pattern recognition protein genes ↑ Antimicrobial molecule ↑ Hexosamine biosynthesis pathway ↑ UDP-N-acetylglucosamine-peptide N-acetylglucosaminyltransferase ↑	(Chien et al. 2020)
Bacillus sp. DDKRC1	Isolated from the gut of Asian seabass (*Lates calcarifer*)	0, 2.94 × 10^7 CFU/100 g feed and diet fermented with *Bacillus sp.* DDKRC1 42 days	Tiger shrimp (*Penaeus monodon*) 2.73 ± 0.01 g	Total heterotrophic count ↑ Amylolytic ↑ Cellulolytic and proteolytic bacterial counts ↑ Phagocytic activity ↑	(De et al. 2018)
Bacillus coagulant	Commercial probiotic	0, 10^5, 10^7 and 10^9 cfu g^{-1} 60 days	Freshwater prawn (*Macrobrachium rosenbergii*) 2.4 ± 0.35 g	Lysozyme activity ↑ Respiratory burst activity ↑	(Gupta et al. 2016)
Bacillus sp. PP9	Isolated from mrigal gut	2 × 10^4, 2 × 10^5 and 2 × 10^6 CFU 60 days	Mrigal (*Cirrhinus mrigala*) 2.5 ± 0.20 g	Hemoglobin percentage ↑ Total erythrocyte count ↑ Total leukocyte count ↑ Corpuscular hemoglobin ↑ Total serum protein ↑ Albumin globulin ratio ↑ Serum bactericidal activity ↑	(Bandyopadhyay et al. 2015)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus subtilis KADR1	Commercial probiotic	10^6; 10^8 and 10^{10} CFU/g 4 weeks	*Labeo rohita*	Serum lysozyme ↑ Phagocytosis ↑ Serum total protein ↑ Respiratory burst ↑ Serum IgM levels ↑ Superoxide dismutase ↑ Alternative complement pathway ↑	(Ramesh & Souissi 2018)
Bacillus subtilis FPTB13 and chitin	Isolated from an indigenous fermented fish product "Shidal"	*B. subtilis* 10^9 cells g^{-1}, chitin 2% and the combition 2 weeks	Labeo catla (*Catla catla*) 40.0 ± 1.9 g	Oxygen radical production ↑ Myeloperoxidase content ↑ Lysozyme activity ↑ Total protein content and alkaline ↑ Phosphatase activity ↑	(Sangma & Kamilya 2015)
B. subtilis, B. licheniformis, and *B. cereus*		0; 1 × 10^5 cfu/g of *B. subtilis;* 1 × 10^5 cfu/g of *B. subtilis* and *B. licheniformis and* 1 × 10^5 cfu/g of *B. subtilis*, *B. licheniformis*, and *B. cereus* 45 days	Common carp (*Cyprinus carpio*) 57.40 ± 0.43 g	Phagocytic percentage ↑ Phagocytic index ↑ Serum immunoglobulin M ↑ Serum lysozyme activity ↑ Intestinal mucosal secretory immunoglobulin A ↑ Peripheral blood lymphocyte proliferation ratio ↑ Superoxide dismutase activity ↑ Glutathione peroxidase activity ↑ Catalase activity ↑ Total antioxidant maleic dialdehyde activity ↑ Glutathione activity ↑	(Wang et al. 2017a)

Bacillus subtilis		10^9 CFU/g 60 days	Gibel carp (*Carassius auratus gibelio*) 60.51 ± 0.51 g	Protective effects against lead toxicity ↑ Superoxide dismutase ↑ Catalase and glutathione ↑ Lysozyme and IgM levels ↑ immune-related genes ↑	(Yin et al. 2018)
Bacillus amyloliquefaciens		0, 10^5, 10^7 and 10^9 CFU/g 70 days	Roho labeo (*Labeo rohita*) 20.23 g	Serum protein and globulin ↑ Albumin, lysozyme, and IgM ↑ Malondialdehyde ↑ Catalase, and superoxide dismutase ↑ Serum aspartate transaminase ↑ Serum alanine transaminase activity ↑ Liver malondialdehyde level ↑	(Nandi et al. 2018)
Bacillus subtilis and β-glucan	Commercial probiotic	1 g kg^{-1} β-glucan and 1×10^9 CFU kg^{-1} *B. subtilis* 70 days	Pengze crucian carp (*Carassius auratus* var. Pengze) 12.89 ± 0.04 g	Acid phosphatase activity ↑ Alkaline phosphatase activity ↑ Glutathione peroxidase activity ↑ Glutathione activity ↓ Catalase activity ↓ Total superoxide dismutase activity ↓	(Cao et al. 2019)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus amyloliquefaciens FPTB16		10^7, 10^8 and 10^9 cells g^{-1} diet 4 weeks	Indian major carp (*Catla catla*) 25.98 ± 2.57 g	Oxygen radical production ↑ Serum lysozyme activity ↑ Total serum protein content ↑ Myeloperoxidase activity ↑ Alkaline phosphatase activity ↑ Expression of IL-1β, TNF-α, C3 and iNOS ↑ IFN-γ expression ↓	(Singh et al. 2017)
Bacillus subtilis	Isolated from the gut of grass carp	0, high-fat diet, and high-fat diet + *B. subtilis* (1 × 10^7 CFU g^{-1}) for 8 weeks	Grass carp (*Ctenopharyngodon idellus*) 50.24 ± 1.38 g	Serum low-density lipoprotein cholesterol ↑ Aspartate aminotransferase ↑ Hepatic mRNA expression of fatty acid synthase ↓ Carnitine palmitoyl transferases ↑ Glutathione ↑ Hydrogen peroxide (H_2O_2) ↓ Malondialdehyde (MDA) contents ↓	(Zhao et al. 2020)
Bacillus licheniformis	Isolated from grass carp	1 × 10^5 cfu/g and 1 × 10^6 cfu/g 56 days	Grass carp (*Ctenopharyngodon idella*) 16.5 g	Superoxide dismutase (SOD) activity ↑ Malondialdehyde (MDA) levels ↓ Antioxidant enzymes *MnSOD* ↑ Catalase (CAT) in the intestine ↑ Proinflammatory cytokines ↓ Anti-inflammatory cytokine ↑ *ZO-1*, *occludin*, and *claudin-c* ↑	(Qin et al. 2020)

B. subtilis YB-1 and *B. cereus* YB-2	Commercial probiotic	0, 10^7 and 10^{10} cfu/g diet 32 days	Sea cucumber (*Apostichopus japonicus*) 50 ± 0.5 g	Phagocytic activity ↑ Superoxide anion production ↑ Lysozyme activity ↑ Catalase activity ↑ Phenoloxidase activity ↑	(Li et al. 2015)
Bacillus baekryungensis MS1	Isolated from a sea cucumber pond in winter	0 and 10^7 CFU/ml 60 days	Sea cucumber (*Apostichopus japonicus*) 4.17 g ± 0.22 g	Superoxide dismutase activity ↑ Catalase activity ↑ Alkaline phosphatase activity ↑ Acid phosphatase activity ↑ Nitric oxide synthetase activity ↑ Phagocytosis and respiratory burst ↑ Ubiquitin-mediated proteolysis pathway ↑	(Liu et al. 2020)
Bacillus cereus EN25	Isolated from mud of sea cucumber culturing water bodies	0, 10^5, 10^7 and 10^9 CFU/g for 30 days	Juvenile sea cucumber (*Apostichopus japonicus*) 0.375 ± 0.024 g	Total coelomocytes count → Acid phosphatase activity → Phagocytosis activity ↑ Respiratory burst activity ↑ Total nitric oxide synthase activity ↑ Superoxide dismutase activity →	(Zhao et al. 2016)
B. amyloliquefaciens 54A and B. *pumilus* 47B	Isolated from gut of striped catfish	1×10^8, 3×10^8, and 5×10^8 CFU g^{-1} feed 90 days	Striped catfish (*Pangasianodon hypophthalmus*)	Phagocytic activity ↑ Respiratory bursts ↑ Lysozyme activity ↑	(Truong Thy et al. 2017)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus aerius B81e	Isolated from healthy hybrid catfish	0 and 10^7 CFU g^{-1} feed 60 days	Basa fish (*Pangasius bocourti*) 69 g	Serum lysozyme activity ↑ Bactericidal activity ↑ Alternative complement activity ↑ Phagocytic activity ↑ Respiratory burst activity ↑	(Meidong et al. 2018)
B. subtilis, B. amyloliquefacien, B. cereus and a commercial B. amyloliquefaciens	Isolated from the intestine of African catfish	10^{10} CFU/ml 60 days	African catfish (*Clarias gariepinus*) 75.23 ± 1.6 g	Lysozyme activity ↑ Nitric oxide and IgM ↑ Myostatin cDNA levels ↑ PACAP expression ↑	(Reda et al. 2018)
Bacillus subtilis WB60 and *Lactobacillus plantarum* KCTC3928	Commercial probiotic	0; *B. subtilis* at 10^6, 10^7, 10^8 and *L. plantarum* at 10^6, 10^7, 10^8 CFU/g diet	Japanese eel (*Anguilla japonica*) 8.29 ± 0.06 g	Lysozyme activity ↑ Superoxide dismutase (SOD) ↑ Myeloperoxidase (MPO) ↑ Level of intestine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ↑ Heat shock protein 70, 90 ↑ Immunoglobulin (IgM) ↑	(Lee et al. 2017)
Bacillus subtilis WB60 and mannanoligosaccharide (MOS)	Commercial probiotic	BS: 0.0, 0.5, and 1.0×10^7 CFU/g diet and MOS: 0 and 5 g/kg diet. 8 weeks	Japanese eel (*Anguilla japonica*) 9.00 ± 0.11 g	Nonspecific enzymatic activities ↑ Heat shock protein 70 mRNA levels ↑ Immunoglobulin M expressions ↑	(Lee et al. 2018)
Bacillus subtilis or *licheniformis*) and (mannan or fructo oligosaccharide)		0, Probiotics (1.0×10^8 CFU/g diet) and prebiotics (5 g/kg diet) 12 weeks	Japanese eel (*Anguilla japonica*) 12.8 ± 0.47 g	Expression of heat shock protein 70 ↑ Expression of immunoglobulin M ↑	(Park et al. 2020)

B. subtilis E20	Commercial probiotic	0, 10^8, 10^9, and 10^{10} CFU kg^{-1} 56 days	Parrotfish (*Oplegnathus fasciatus*)	Lysozyme activity ↑ Respiratory burst ↑ Phagocytic activity ↑	(Liu et al. 2018)
B. velezensis V4 and *Rhodotorula mucilaginosa* compound	Isolated from the water of RAS rearing salmonid	0; *B. velezensis* V4 5×10^6, *R. mucilaginosa* 5×10^7 (CFU g^{-1}), (*B. velezensis* V4 1.5×10^7, *R. mucilaginosa* 1.5×10^8 (CFU g^{-1}), and *B. velezensis* V4 2.5×10^7, *R. mucilaginosa* 2.5×10^8 (CFU g^{-1}) 62 days	Juvenile Atlantic salmon (*Salmo salar L.*) 180.18 ± 3.64 g	Acid phosphatase ↑ IgM ↑ Nitric oxide ↑ Glutamic pyruvic transaminase ↑ Glutamic oxalacetic transaminase ↑ Lysozyme ↑ Total superoxide dismutase malondialdehyde ↑ Glutathione ↑ Glutathione peroxide ↑ Total antiodant capacity ↑ Malondiadehyde ↑	(Wang et al. 2019a, 2019b)
Bacillus subtilis	Commercial probiotic	0, 1×10^4, 1×10^6, 1×10^8 and 1×10^{10} CFU kg^{-1} diet 60 days	Red sea bream (*Pagrus major*) 3.99 ± 0.01 g	Hematocrit and hemoglobin values ↑ Nitro blue tetrazolium value ↑ Serum bactericidal activity ↑ Serum lysozyme activity ↑ Serum peroxidase activity ↑ Catalase activity ↑	(Zaineldin et al. 2018)
B. subtilis and *B. licheniformis*	Commercial probiotic	0.6 g/kg 84 days	Turbots (*Scophthalmus maximus*) 95.8 ± 17.7 g	Plasma lysozyme activity → Neutrophil reactive oxygen species (ROS) → Production, and total plasma protein levels → Plasma glucose and triglyceride ↑ Glucose levels ↑ Cortisol levels ↓	(Fuchs et al. 2017)

(continued)

Table 1.2 (continued)

Species	Source	Doses and duration	Fish species	Parameters investigated	References
Bacillus sp. SJ-10 plus β-glucooligosaccharides	Identified from traditional Korean fermented fish	0; 1 × 10^8 CFU g^{-1} BSJ-10; 0.1% BGO, and 1 × 10^8 CFU g^{-1} BSJ-10 + 0.1% BGO. 8 weeks	Olive flounder (*Paralichthys olivaceus*) 10 ± 0.25 g	Respiratory burst activity ↑ Superoxide dismutase ↑ Lysozyme activity ↑ Expression of interleukin (IL)-1β ↑ Tumor necrosis factor (TNF)-α ↑	(Hasan et al. 2018)
Bacillus amyloliquefaciens R8		0 and 92 x 10^6 CFU g^{-1} 30 days	Zebrafish (*Danio rerio*) 0.48 g	Xylanase activity ↑ mRNA expressions of glycolysis-related genes ↑ Enzyme activities ↑ Expression of innate immune-related genes ↑ Expressions of oxidative stress-related genes ↑	(Lin et al. 2019)
Bacillus pumilus SE5		0 and 1.0 × 10^8 CFU g^{-1} 60 days	Grouper (*Epinephelus coioides*) 14.6 ± 0.2 g	Phagocytic activity ↑ Serum complement C3 and IgM levels ↑ SOD activity ↑ Expression of TLR2 and pro-inflammatory cytokines ↑	(Yan et al. 2016)
Bacillus amyloliquefaciens (GB) and Yarrowia lipolytica lipase 2 (YLL2)	Commercial probiotic	0, 5.0 g/kg GB-9, 4.0 g/kg YLL2, and 5.0 g/kg GB-9 + 4.0 g/kg YLL2 12 weeks	Hybrid sturgeon (*Acipenser schrenkii* ♂and *Acipenser baeri* ♀) 5.0 g	Skin mucus lysozyme activity ↑ Leukocytes phagocytosis activity ↑ Reactive oxygen species level ↑ Alternative complement pathway activity ↑ Peroxidase and lysozyme activity ↑	(Fei et al. 2018)

Bacillus subtilis and *Bacillus cereus toyoi*	Commercial probiotic	0, 6 × 10^3 and 1.5×10^6 CFU g^{-1} of diet. 9 and 20 weeks	Rainbow trout (*Oncorhynchys mykiss*) and brown trout (*Salmo trutta*) 15.6 g	Plasma lysozyme activity ↑ Alternative complement activity ↑ Peroxidase activity ↑	(Ramos et al. 2017)
Bacillus amyloliquefaciens-JFP2		0 and 1.4×10^6 (CFU/g) of feed 90 days	Rock Bream (*Oplegnathus fasciatus*) 25.4 ± 0.13 g	Serum antioxidant and lysozyme activity ↑ Triglyceride and total cholesterol ↓ Alanine aminotransferase ↑ Aspartate aminotransferase ↑	(Kim et al. 2017)
BetaPlus® (*B. subtilis*, *B. licheniformis* and Isomaltooligosaccharides	Commercial probiotic	0 and 2 g kg^{-1} IMOS + 1 g kg^{-1} BetaPlus® in 7 weeks	Caspian Brown Trout (*Salmo trutta caspius*) 9 g	Immunoglobulin M levels ↑ Alanine aminotransferase activity ↑ Lactate dehydrogenase activity ↑	(Aftabgard et al. 2019)
Bacillus licheniformis	Commercial probiotic	10^3, 10^5, and 10^7 CFU/mL 8 weeks	Abalone (*Haliotis discus* hannai Ino.) 4.17 ± 0.32 g	Blood lymphocytes ↑ Activity of acid phosphatase ↑ Expression level of heat shock protein 70 ↑ Phagocytic activity ↑ Myeloperoxidase and catalase ↑ Expression levels of CAT ↑ Expression of thioredoxin ↑ Superoxide dismutase (SOD) ↑ Respiratory burst of blood lymphocytes ↑ Expression levels of Mn-SOD ↑	(Gao et al. 2018)

1.4.1 Tilapias

Selim and Reda (2015) found that *Bacillus amyloliquefaciens* spores supplementation at concentrations of 1×10^6 (G3) and 1×10^4 (G2) colony-forming units per gram (CFU g^{-1}) of feed significantly enhanced serum killing, serum nitric oxide, serum lysozyme activities, as well as IL-1 and TNF α mRNA levels in the kidneys of Nile tilapia, *O. niloticus*. The cell wall components of both Gram-positive and Gram-negative bacteria are able to stimulate cytokine production (Henderson et al. 1999). Probiotic bacteria colonize in the gut and are involved with the gut-associated lymphoid tissue to stimulate systemic signals that end with cytokine production (Kesarcodi-Watson et al. 2008; Rangavajhyala et al. 1997; Rescigno et al. 2001; Ringø 2011). Similarly, dietary inclusion of *B. subtilis* singularly or *B. subtilis* combined with *S. cerevisiae* and *A. oryzae*; *B. subtilis* with *B. licheniformis*; *B. subtilis* and *Bacillus licheniformis* (BS) combined with traditional Chinese medicine (TCM), and *B. subtilis* with Aqua NZ and AP193 significantly enhanced innate immune response, growth, relative immune, and antioxidant gene expressions of Nile tilapia (Abarike et al. 2018a; Abarike et al. 2018b; Addo et al. 2017a, 2017b; Iwashita et al. 2015; Liu et al. 2017; Wang et al. 2020a). Dietary inclusion of *B. licheniformis* has been found to increase alkaline phosphatase, myeloperoxidase, lysozyme, reactive oxygen species, reactive nitrogen species, superoxide dismutase, and glutathione peroxidase of Mozambique tilapia (*Oreochromis mossambicus*) (Gobi et al. 2018). Also, supplementation of *Bacillus licheniformis* HGA8B significantly improved lysozyme activity and content of complement C3 (Han et al. 2015). It is well documented that, the immune system can be nonspecifically modulated by probiotics (Hoseinifar et al. 2015; Lazado and Caipang 2014; Nayak 2010). Moreover, colony formation and adhesion of probiotics in the intestine of fish are necessary to enhance the immune responses (Ausubel 2005). Interaction between probiotic cells and immune system are through microbe associated molecular patterns (MAMPs) consisting of specific cell wall polysaccharides, peptidoglycan, lipoprotein anchors, and lipoteichoic acids (Hosoi et al. 2003). Cells or components of immune system can interact with MAMPs by pattern recognition receptor such as toll-like receptors, C-type receptor, and nucleotide oligomerization domain-like receptors (Bron et al. 2012; Kleerebezem et al. 2010). This fact may indicate that, addition of fresh culture of *B. licheniformis* to the diet maintains a high level of probiotics in the diet and improve the immune responses in fish. Similar results have been reported in Nile tilapia fed *B. cereus* and *B. pumilus* (Srisapoome and Areechon 2017; Wang et al. 2017b).

1.4.2 Shrimps

In shrimp, *B. licheniformis* has been intensively applied in Pacific white shrimp (*Litopenaeus vannamei*). Amoah et al. (2019) indicated that dietary inclusion of 1×10^8 CFU g^{-1} feed significantly enhanced activity of lysozyme (LYZ), acid phosphatase (ACP), superoxide dismutase (SOD), total protein (TP), albumin (ALB) in serum, glutathione peroxidase (GSH-Px) in serum and liver of Nile tilapia. Similarly, dietary administration of *B. licheniformis* significantly upregulated the expression of catalase, glutathione peroxidase, superoxide dismutase (SOD), penaeidin-3a (Pen-3a), and heat shock protein (Hsp-70) genes of Pacific white shrimp, *Litopenaeus vannamei* (Chen et al. 2020a, 2020b). In addition, the combination of *B. licheniformis* with *B. subtilis* significantly enhanced lysozyme and hemocyte cell count and upregulated the expression of proPO, LvToll1 and SOD, Hsp70, and TGase genes (Sadat Hoseini Madani et al. 2018; Sánchez-Ortiz et al. 2016). Likewise, dietary inclusion of *B. subtilis* E20 singularly or combined with other probiotics significantly innate immune response and related immune gene expression of Pacific white shrimp, *Litopenaeus vannamei* (Chien et al. 2020; Won et al. 2020a). Also, Khademzade et al. (2020) reported that dietary inclusion of *Bacillus cereus* and *Pediococcus acidilactici* significantly enhanced total hemocyte count, total protein, and lysozyme activities of *L. vannamei*. Similar results were found in tiger shrimp and freshwater pawn fed *Bacillus* sp. and *Bacillus coagulant* where significant increase in total heterotrophic count, amylolytic, cellulolytic, and proteolytic bacterial counts, phagocytic, lysozyme, and respiratory burst activities was recorded (De et al. 2018; Gupta et al. 2016). At molecular levels, Sánchez-Ortiz et al. (2016) indicated that dietary supplementation of *Bacillus* spp. resulted in upregulation of proPO, LvToll1, SOD genes, except the TGase gene expression. Similarly, Tepaamorndech et al. (2019) revealed that dietary inclusion of *Bacillus aryabhattai* TBRC8450 significantly upregulated C-type lectin, penaeidin-3, and heat shock protein 60 genes, as well as enhanced thioredoxin, ferritin, phenoloxidase, and total antioxidant activities of Pacific white shrimp, *Litopenaeus vannamei*. However, no significant increase in total hemocyte count, and superoxide dismutase were observed (Tepaamorndech et al. 2019).

1.4.3 Carps

In mrigal, *Cirrhinus mrigala*, Bandyopadhyay et al. (2015) indicated that dietary inclusion of *Bacillus* sp. PP9 significantly improved hemoglobin percentage, total erythrocyte count, total leukocyte count, corpuscular hemoglobin, total serum protein, albumin globulin ratio, and serum bactericidal activity. Similarly, dietary supplementation of *B. subtilis* singularly or combined with other *Bacillus* sp. and prebiotics significantly stimulated hematological, antioxidant, and immunological parameters of *Labeo rohita* (Ramesh and Souissi 2018); Labeo catla, *Catla catla* (Sangma and Kamilya 2015); common carp, *Cyprinus carpio* (Wang et al. 2017a); grass carp, *Ctenopharyngodon idellus* (Zhao et al. 2020), and Pengze crucian carp, *Carassius auratus* var. Pengze (Cao et al. 2019). At gene level, Yin et al. (2018) found that supplementation of *B. subtilis* resulted in higher protective effects against lead toxicity, superoxide dismutase, catalase and glutathione, lysozyme and IgM levels, as well as immune-related genes of gibel carp, *Carassius auratus gibelio*. Likewise, dietary inclusion of *B. amyloliquefaciens* significantly stimulated innate immune response, antioxidant, and relative immune gene expressions of roho labeo,

Labeo rohita (Nandi et al. 2018); Indian major carp, *Catla catla* Singh et al. (2017), and grass carp, *Ctenopharyngodon idella* (Qin et al. 2020).

1.4.4 Sea Cucumber (*Apostichopus japonicus*)

Supplementation of *B. cereus* singularly or combined with *B. subtilis* significantly enhanced total coelomocytes count, acid phosphatase, phagocytosis, respiratory burst, total nitric oxide synthase, catalase, phenoloxidase, and superoxide dismutase activities (Li et al. 2015). Recently, Liu et al. (2020) indicated that dietary administration of *B. baekryungensis* significantly enhanced superoxide dismutase, catalase, alkaline phosphatase, acid phosphatase, nitric oxide synthetase, phagocytosis, respiratory burst activities, and ubiquitin-mediated proteolysis pathway. Ubiquitin-mediated proteolysis plays an important role in the dynamic regulation of host defense against pathogen infection. It has been reported that a number of key joint molecules in the natural immune and antiviral signaling pathways can be modified by ubiquitination to regulate the antiviral immune response of the body (Chuang and Ulevitch 2004; Liu and Chen 2011). Ubiquitination plays an important role in the Toll-like receptor (TLR) signaling pathway. The activation of this pathway leads to the upregulated expression of Toll-like receptors and enhances nonspecific immunity (Bhoj and Chen 2009). The upregulation of *TLR* in this study is consistent with the above theory. In the immune system, mTOR signaling plays an important role in maintaining immune homeostasis, for example, the survival and migration of natural immune cells and the secretion of inflammatory factors (Katholnig et al. 2013; Weichhart et al. 2008). Studies have found that the mTOR signaling pathway negatively regulates nonspecific immune responses (Weichhart et al. 2008). Therefore, the downregulation of the mTOR pathway in sea cucumber is beneficial to improve sea cucumber immunity.

1.4.5 Catfish

In striped catfish, *Pangasianodon hypophthalmus*, Truong Thy et al. (2017) reported that dietary inclusion of *B. amyloliquefaciens* and *B. pumilus* significantly enhanced phagocytic, respiratory bursts, and lysozyme activities. Similar results were observed in basa fish, *Pangasius bocourti* fed *B. aerius* (Meidong et al. 2018). Likewise, combination of *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, and *B. amyloliquefaciens* (Reda et al. 2018).

1.4.6 Japanese eel

Dietary inclusion of *B. subtilis* and *Lactobacillus plantarum* significantly enhanced lysozyme, superoxide dismutase (SOD), myeloperoxidase (MPO), level of intestine glyceraldehyde-3-phosphate dehydrogenase (GAPDH), heat shock protein 70, 90, and immunoglobulin (IgM). Similarly, dietary inclusion of *B. subtilis* and mannanoligosaccharide (MOS) significantly improved nonspecific enzymatic activities, heat shock protein 70 mRNA levels, and immunoglobulin M expressions (Lee et al. 2018). More recently, Park et al. (2020) indicated that dietary inclusion of *B. subtilis* or *B. licheniformis* and mannan or fructo oligosaccharide upregulated heat shock protein 70 and immunoglobulin M genes.

1.4.7 Other Species

Dietary inclusion of *B. subtilis* singularly or combined with *B. licheniformis*, *Bacillus cereus toyoi*, and isomaltooligosaccharides significantly stimulated hematological, innate immune response, antioxidant, and gene expression of parrotfish, *Oplegnathus fasciatus* (Liu et al. 2018); red sea bream, *Pagrus major* (Zaineldin et al. 2018); turbots, *Scophthalmus maximus* (Fuchs et al. 2017); rainbow trout, *Oncorhynchus mykiss* and brown trout, *Salmo trutta* (Ramos et al. 2017), and Caspian brown trout, *Salmo trutta caspicus* (Aftabgard et al. 2019). Regarding *B. amyloliquefaciens*, dietary inclusion of *B. amyloliquefaciens* singularly or combined with Yarrowia lipolytica lipase 2 (YLL2), *B. licheniformis* significantly enhanced innate immune response, antioxidant, and gene expression of rock bream, *Oplegnathus fasciatus* (Kim et al. 2017); hybrid sturgeon, *Acipenser schrenckii* ♂and *Acipenser baerii* ♀ (Fei et al. 2018), and zebrafish, *Danio rerio* (Lin et al. 2019).

In juvenile Atlantic salmon (*Salmo salar* L.), Wang et al. (2019a) reported that *B. velezensis* V4 and *Rhodotorula mucilaginosa* compound led to an increase in acid phosphatase, IgM, nitric oxide, glutamic pyruvic transaminase, glutamic oxalacetic transaminase, lysozyme, total superoxide dismutase malondialdehyde, glutathione, glutathione peroxide, total antioxidant capacity, and malondialdehyde. Similarly, dietary inclusion of *Bacillus licheniformis* significantly enhanced hematological, innate immune response, and Mn-SOD gene expression (Gao et al. 2018). Also, significant increase in innate immune response and relative immune gene expressions were observed in grouper, *Epinephelus coioides,* fed *Bacillus pumilus* (Yan et al. 2016) and in olive flounder, *Paralichthys olivaceus,* fed *Bacillus* sp. SJ-10 plus β-glucooligosaccharides (Hasan et al. 2018).

1.5 Conclusion

This chapter addressed the role of *Bacillus* probiotics in sustainable aquaculture. Although a wide range of researches have indicated beneficial effects of *Bacillus* species on grow rate, immunity, and disease resistance of farmed fish and shellfish, the investigated effects were species specific. In order to evaluate in vivo adherence and colonization of *Bacillus* bacteria within the complex microbial ecosystem of the intestine, detection of green fluorescence protein (GFP) tagged strains or fluorescence in situ hybridization (FISH) targeting 16S rRNA to identify the probiotics on the mucus surface must be carried out. Furthermore, mucus-associated (autochthonous) microbiome must be investigated by next-generation sequencing (NGS), transcriptomic, metagenomics or proteomic profiling, and not the allochthonous microbiome; mostly investigated *per sc*. In addition, we recommend that gnotobiotic approaches are used in future studies, as the gnotobiotic approaches have been reported to have important roles to understand the function of gut microbiota on numerous biological processes of the host. Moreover, data is needed to understand the mechanisms by which the immune system of the intestinal mucosa discriminates between pathogenic, probiotics, and commensal microorganisms.

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