

## SPECIAL ISSUE ARTICLE

# Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture

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## Keywords

bacilli, finfish, health, lactic acid bacteria, probiotics, shellfish.

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2020/0123: received 22 January 2020, revised 23 February 2020 and accepted 26 February 2020

doi:10.1111/jam.14628

## Abstract

Probiotics administration in aquafeed is known to increase feed consumption and absorption due to their capacity to release a wide range of digestive enzymes and nutrients which can participate in digestion process and feed utilization, along with the absorption of diet components led to an increase in host's health and well-being. Furthermore, probiotics improve gut maturation, prevention of intestinal disorders, predigestion of antinutrient factors found in the feed ingredients, gut microbiota, disease resistance against pathogens and metabolism. The beneficial immune effects of probiotics are well established in finfish. However, in comparison, similar studies are less abundant in the shellfish. In this review, the discussions will mainly focus on studies reported the last 2 years. In recent studies, native probiotic bacteria were isolated and fed back to their hosts. Although beneficial effects were demonstrated, some studies showed adverse effects when treated with a high concentration. This adverse effect may be due to the imbalance of the gut microbiota caused by the replenished commensal probiotics. Probiotics revealed greatest effect on the shrimp digestive system particularly in the larval and early post-larval stages, and stimulate the production of endogenous enzymes in shrimp and contribute with improved the enzyme activities in the gut, as well as disease resistance.

## Introduction

For many years, antibiotics and chemotherapeutics were supplemented in animals' diets at subtherapeutic levels, to promote benefits by enhancing growth rate, reducing mortality and improving reproductive performance. In 2003, the European Union stated in Regulation (EC) No. 1831/2003; 'Antibiotics, other than coccidiostats or histomonostats, shall not be authorized as feed additives'. Consequently, this banning urgently made the scientific community to seek for alternatives to reduce the abuse of antibiotics, and one of the promising feed

additive was probiotic. Probiotics/fermented milk has a very long history as Genesis 18:8 stated, New Living Translation; 'When the food was ready, Abraham took some yogurt and milk and the roasted meat, and he served it to the men. As they ate, Abraham waited on them in the shade of the trees'. According to Bottazzi (1983), the Roman historian Plinius in 76 BC recommended administration of *fermented milk products* for treating gastroenteritis. However, the modern history of probiotics started more than a century ago, as the Russian Nobel prizewinner, Elie Metchnikoff, performed the observation that the regular consumption of some

fermented milk products containing viable bacterial species may have a beneficial role in the maintenance and reestablishment of microbiota and consequently intestinal homeostasis. The term probiotics, 'to be used for substances that favours the growth of micro-organisms' was first proposed by Lilly and Stillwell (1965), but more recently, Hill *et al.* (2014) suggested a more correct definition of probiotics 'live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host'.

Since the first application of probiotics in aquaculture was published by Kozasa (1986) and the first review discussing probiotics by Ringø and Gatesoupe (1998), have several comprehensive reviews been published (e.g. Gatesoupe 1999; Merrifield *et al.* 2010; Hai 2015; Hoseinifar *et al.* 2018; Ringø *et al.* 2018; Ringø 2020). Of bacteria mostly used as probiotics in aquaculture are, lactic acid bacteria and *Bacillus*, but several other genera such as *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodospiridium*, *Roseobacter*, *Streptomyces* and *Vibrio*, and microalgae (*Tetraselmis*) and yeast (*Debaryomyces*, *Phaffia* and *Saccharomyces*) are also used.

Probiotic administrations mainly depends on several factors, that is the probiotics, supplementation form, vector of administration, dosage level and duration of application and several different administration modes have been used: oral administration via diet or water/bath, administration of several probiotics in combination, inactivated bacteria, spores, administration—continuously or regular intervals, and co-administration of probiotics with prebiotics (synbiotics) or plant products. Important questions to be clarified when discussing probiotics are; species isolated from the host, host specificity *vs* strains from other species or commercial probiotics, as well as single or combined administration.

The mechanisms of actions of probiotics in aquaculture are divided into; antagonistic compound secretion, substances produced by probiotics; act as antagonist for quorum sensing mechanism, adhesion and colonization to the intestinal mucosa, competitive exclusion when probiotic bacteria colonize the intestine and thereby inhibiting adherence and colonization of pathogenic bacteria, improved functionality of the gastrointestinal (GI) tract, modulation of the GI tract microbiota, competition for iron, sources of nutrients and enzymes for digestion, enhancement of immune responses, antiviral effect and improve water quality through modulation of the water microbiota.

In order to avoid overlaps with previous review papers, the current review aimed to present an updated overview of recently published data, mainly from 2018 and 2019, on health benefits of LAB and *Bacillus* probiotics, on

their effect on growth performance, modulation of the gut microbiota, the immune system and disease resistance in finfish and shellfish.

## Methods of probiotic administration

To our knowledge, the first application of probiotics in aquaculture was carried out by Kozasa (1986), but since then the environment-friendly treatment has increased rapidly, and several comprehensive aquaculture reviews have been published (e.g. Gatesoupe 1999; Merrifield *et al.* 2010; Hai 2015; Hoseinifar *et al.* 2018; Ringø *et al.* 2018, 2020; Ringø 2020). However, it is essential to investigate the best way of administration, optimal dose, and the technical solutions required, especially to keep the probiotics alive in dry pellets (Gatesoupe 1999).

Probiotic administrations depends on several factors i.e. the probiotics used, supplementation form, vector of administration, dosage level and duration of application, and several different administration modes are proposed:

- i Oral administration via diet or water/bath. Inclusion to the diet is the most widely used administration method. Probiotics and cell wall components (parabiotics) are applied in the feed, added to the entire tank or pond water to confer protection against infection. In fish- and shellfish larvae, live food (e.g. *Artemia*) has revealed to be an efficient carrier of probiotics.
- ii Administration of several probiotics in combination. In the review, "Probiotics in man and animals," Fuller (1989) wrote, "Probiotic preparations may consist of single strains or may contain any number up to eight strains." However, since the early 1990s most aquaculture studies used single administration, but during the last years, supplementation of multiple probiotics in the diets has gained interest. The advantage of multiple-strain preparations is; they are active against wider range of conditions and species.
- iii Inactivated bacteria. For example, oral administration of heat- inactivated *Lactobacillus delbrueckii* and *Bacillus subtilis*, individually or combined.
- v Spores help the bacteria to survive by being resistant to extreme changes in the bacteria's habitat including extreme temperatures, lack of moisture/drought, or being exposed to chemicals and radiation. Bacterial spores can also survive at low nutrient levels, and spore-forming probiotic bacteria have received increased scientific and commercial interest.

- v Culturing, storing and administration. Probiotics are usually added to feed as freeze-dried cultures, and sometimes mixed with lipids to be added as top.
- vi Lyophilization or freeze drying, is a low temperature dehydration process, involving freezing of the product at low pressure, and removing the ice by sublimation. This method is used in probiotic studies of finfish and shellfish.
- vii Administration – continuously or regular intervals? Most studies carried out have continuously fed the host fish for a wide range of time, varying from 15 to 94 days (Hai 2015). The continual application of LAB, bacilli, and certain Gram-negative bacteria increase colonization of the supplemented bacteria, and modulated the microbial population in the GI tract. However, an important question arises; are the probiotics permanently colonisers in the GI tract?
- viii Co-administration of probiotics with prebiotics or plant products.

Important questions when discussing probiotics are; species isolated from the host, *vs.* strains isolated from other species or commercial probiotics?

### LAB as probiotics in finfish and shellfish

#### Improve feed utilization

Numerous investigations have recently conducted the alternation of enzyme patterns as a consequence of the consumption of LAB in shellfish and finfish (Tables 1 and 2). Recently, dietary inclusion of *Lactobacillus* sp. and *Lb. pentosus* at concentrations of  $10^7$  and  $5 \times 10^8$  CFU per g improved several digestive enzymes of Pacific white shrimp (*Litopenaeus vannamei*) (Du *et al.* 2019; Zuo *et al.* 2019). Similarly, an elevation in protease, amylase and alkaline phosphatase was observed in narrow clawed crayfish (*Astacus leptodactylus*) fed *Lb. plantarum* at concentrations of  $10^7$ ,  $10^8$  and  $10^9$  CFU per g (Valipour *et al.* 2019). Dawood *et al.* (2019) reported that incorporation of heat-killed *Lb. plantarum* at 50, 100 or 1000 mg kg<sup>-1</sup> significantly enhanced amylase, lipase and protease activity of Nile tilapia (*Oreochromis niloticus*). Significant increase in lipase, amylase, trypsin, alkaline phosphatase and protease activity also recorded in common carp (*Cyprinus carpio*), olive flounder (*Paralichthys olivaceus*) and rainbow trout (*Oncorhynchus mykiss*) fed LAB in combination with  $\beta$ -glucan, mana oligosaccharide, *Bacillus* sp. and *Citrobacter* (Jang *et al.* 2019; Mohammadian *et al.* 2019a, 2019b).

#### Promote growth performance

Probiotic is one of the most promising means to sustain the normal growth, health and well-being of farmed fish

and shellfish because they serve as nutrients source, vitamins and digestive enzymes, and they will significantly contribute to feed consumption, nutrients uptake and host's growth rate (Nath *et al.* 2019). Probiotics consumption have been speculated to improve the host's appetite or boost organisms' digestibility by stimulating the excretion of digestive enzymes and maintaining the balance of intestinal microbes, which led to the improvement of nutrients absorption and utilization, as well as survival and growth of the host.

Most studies using LAB in shellfish focus on growth performance and survival rate. *Lb. pentosus* and *Lb. plantarum* inclusion in Pacific white shrimp diets significantly improved growth performance and feed utilization (e.g. Correa *et al.* 2018; Gao *et al.* 2018; Zheng *et al.* 2018). Recently, Zuo *et al.* (2019) revealed that supplementation of *Lactobacillus* at  $10^7$  CFU per g for 27 days significantly increased body weight of Pacific white shrimp. In contrast, no significant difference in growth parameters was recorded in narrow clawed crayfish fed *Lb. plantarum* for 97 days (Valipour *et al.* 2019). Incorporation of LAB with other probiotics or functional feed additives resulted in higher growth performance in shellfish. Dietary supplementation of *Enterococcus faecalis* and *Pediococcus acidilactici* significantly improved weight gain and specific growth rate of narrow clawed crayfish and mud crab (*Scylla paramamosain*) (Safari *et al.* 2017; Yang *et al.* 2019). Wang *et al.* (2019) revealed that dietary in combination of *Lb. pentosus*, *Lactobacillus fermentum*, *B. subtilis* and *Saccharomyces cerevisiae* significantly improved growth performance and survival rate of Pacific white shrimp, but no significant difference was revealed in carcass composition.

Most finfish studies focused on the effects of different LAB and combination with other probiotics and natural immunostimulants on growth performance. Dietary administration of *Lactobacillus* spp. at different concentrations significantly enhanced growth parameters of several finfish species (e.g. Abdelfatah and Mahboub 2018; Alishahi *et al.* 2018; Dawood *et al.* 2019; Feng *et al.* 2019; Jami *et al.* 2019; Van Nguyen *et al.* 2019). The administration of *P. acidilactici* revealed significant improved growth performance of several finfish species (e.g. Tardashti *et al.* 2017; Rahimnejad *et al.* 2018; Ashouri *et al.* 2018; Hoseinifar *et al.* 2019). Dietary inclusion of *Lb. plantarum* in combination with orange peel derived pectin, corncob-derived xylooligosaccharide, *Cordyceps militaris* or *Bacillus velezensis* significantly enhanced growth performance of Nile tilapia (Van Doan *et al.* 2017, 2018, 2019, 2020a). Similarly, dietary administration of *Lactobacillus* in combination with  $\beta$ -glucan or mananoligosaccharide significantly stimulated the growth performance and feed utilization of common carp (Mohammadian

**Table 1** Effect of lactic acid bacteria on growth performance and disease resistance in shellfish

Species	Isolated from	Doses and duration	Shellfish species	Parameters investigated	References
<i>Lactobacillus plantarum</i>	Commercial probiotic	10 <sup>9</sup> CFU per ml 45 days	<i>Litopenaeus vannamei</i>	↑ FW, WG, SGR, resistance against the stress of acute low salinity ↓ FCR	Zheng <i>et al.</i> (2017)
<i>Lb. plantarum</i>	Shrimp intestine	10 <sup>7</sup> CFU per ml 35 days	<i>L. vannamei</i>	→ Growth performance, water quality	Correa <i>et al.</i> (2018)
<i>Lb. plantarum</i>		20 × 10 <sup>3</sup> cells per ml and 10 <sup>8</sup> (CFU) per ml	<i>L. vannamei</i>	↑ Water quality in biofloc system ↓ Reduce shrimp diseases and environmental impact	Pacheco-Vega <i>et al.</i> (2018)
<i>Lb. plantarum</i>	Commercial probiotic	10 <sup>9</sup> CFU per ml 15 days	<i>L. vannamei</i>	↑ Growth performance, digestive enzyme activities, enterocytes height	Zheng <i>et al.</i> (2018)
<i>Lb. plantarum</i>	Isolated from rainbow trout intestine	10 <sup>7</sup> , 10 <sup>8</sup> and 10 <sup>9</sup> CFU per g 97 days	<i>Astacus leptodactylus</i>	↑ Protease, amylase, alkaline phosphatase → FW, WG, SGR, SR	Valipour <i>et al.</i> (2019)
<i>Lactobacillus pentosus</i>		0 (control), 10 <sup>6</sup> , 10 <sup>7</sup> and 10 <sup>8</sup> CFU g <sup>-1</sup> 28 days	<i>L. vannamei</i>	↑ Growth performance, feed utilization, digestive enzyme activities, resistance against <i>Vibrio vulnificus</i> , <i>Vibrio rotiferianus</i> and <i>Vibrio campbellii</i>	Zheng and Wang (2017)
<i>Lb. pentosus</i>	Intestinal tract of abalone	10 <sup>3</sup> , 10 <sup>5</sup> and 10 <sup>7</sup> CFU per g 8 weeks	<i>Haliotis discus hannai</i>	↑ SR, food intake, shell length-specific growth rate, antioxidant capacity, resistance against <i>Vibrio parahaemolyticus</i> ↓ FCR	Gao <i>et al.</i> (2018)
<i>Lb. pentosus</i>	Gut of <i>Chaeturichthys stigmatias</i>	5 × 10 <sup>8</sup> CFU g feed <sup>-1</sup> 4 weeks	<i>L. vannamei</i>	↑ Digestion related enzymes, resistance against <i>V. parahaemolyticus</i> , induced stress response genes expression	Du <i>et al.</i> (2019)
<i>Lactobacillus</i>	Intestine of <i>L. vannamei</i>	10 <sup>7</sup> CFU per g 27 days	<i>L. vannamei</i>	↑ Body weight, digestive enzymes, resistance against WSSV	Zuo <i>et al.</i> (2019)
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Intestine, <i>L. vannamei</i>	10 <sup>6</sup> , 10 <sup>7</sup> and 10 <sup>8</sup> CFU per g	<i>L. vannamei</i>	↑ Growth performance, activities of digestive enzymes, <i>Lactobacillus</i> and <i>Bacillus</i> counts, resistance against <i>Vibrio anguillarum</i> , <i>Vibrio</i> counts	Adel <i>et al.</i> (2017a)
<i>Pediococcus pentosaceus</i>	Intestine of <i>L. vannamei</i>	0, 10 <sup>6</sup> , 10 <sup>7</sup> and 10 <sup>8</sup> CFU per g diet 8 weeks	<i>L. vannamei</i>	↑ Growth performance, protease and amylase activities, <i>Lactobacillus</i> sp. and <i>Bacillus</i> sp. intestinal counts	Adel <i>et al.</i> (2017b)
<i>Lb. plantarum</i> and <i>Lac. lactis</i>	Isolated from bee gut	2–4 × 10 <sup>8</sup> CFU per g 16 days	<i>L. vannamei</i>	↑ Resistance against <i>V. parahaemolyticus</i>	Chomwong <i>et al.</i> (2018)
<i>Enterococcus faecalis</i> and <i>Ent. faecium</i>	Intestine of Prawn and mullet	N/A	<i>L. vannamei</i>	↑ Resistance against <i>Aeromonas hydrophila</i> and <i>V. vulnificus</i>	Cui <i>et al.</i> (2017)
<i>Ent. faecalis</i> and <i>Pediococcus acidilactici</i>	Commercial probiotics	7.86 log CFU per g 126 days	<i>A. leptodactylus</i>	↑ Growth performance, resistance against <i>A. hydrophila</i>	Safari <i>et al.</i> (2017)
<i>Lb. pentosus</i> , <i>Lb.</i>				<i>fermentum</i> , <i>B. subtilis</i> , <i>Saccharomyces cerevisiae</i>	Commercial probiotics
10 <sup>7</sup> , 10 <sup>8</sup> and 10 <sup>9</sup> CFU (kg diet) <sup>-1</sup> 56 days	<i>L. vannamei</i>	↑ Growth performance, survival rate, resistance against <i>V. parahaemolyticus</i>	Wang <i>et al.</i> (2019)		

(Continued)

Table 1 (Continued)

Species	Isolated from	Doses and duration	Shellfish species	Parameters investigated	References
<i>Ent. faecalis</i> and <i>P. pentosaceus</i>	N/A	→ Carcass composition 10 <sup>9</sup> CFU per g 6 weeks	<i>Scylla</i> <i>paramamosain</i>	↑ WG, SGR, resistance against <i>V. parahaemolyticus</i>	Yang et al. (2019)

Final weight (FW), 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 shellfish. N/A—no information available; ↑—positive effect; ↓—negative effect; →—no effect.

et al. 2019b). A significant increase in growth rate was also observed in Asian seabass (*Lates calcarifer*) which fed a mixture LAB with *B. subtilis* and yeast (Lin et al. 2017; Niu et al. 2019); sea cucumber (*Apostichopus japonicus*) fed *Lb. plantarum*, *Weissella*, *Lac. lactis* and *Ent. faecalis* (Li et al. 2018); Nile tilapia fed *Lactobacillus rhamnosus* and *Lac. lactis* subsp. *lactis* or Jerusalem artichoke (Xia et al. 2018; Sewaka et al. 2019), and in rainbow trout fed *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Citrobacter* or *Lactobacillus buchneri*, *Lb. fermentum* and yeast (Vazirzadeh et al. 2019; Mohammadian et al. 2019a).

#### Increase disease resistance

Probiotics have been proven as an effective tool for disease prevention in aquaculture (Hoseinifar et al. 2018; Ringø 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, enzymes and/or hydrogen peroxide as well as the alteration of the intestinal pH due to the generation of organic acids. The inhibition of intestinal related diseases has been reported in several cultured species by probiotic incorporation in aquafeeds (e.g. Ringø et al. 2018; Wanka et al. 2018; Serra et al. 2019). 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. *Lb. plantarum* was the most studied probiotic in finfish and shellfish. Dietary supplementation of *Lb. plantarum* significantly increased disease resistance of Pacific white shrimp against *Vibrio* spp. (Pacheco-Vega et al. 2018) and common carp against *Aeromonas hydrophila* (Soltani

et al. 2017). In case of *Lb. pentosus*, dietary inclusion significantly increased disease resistance of Pacific white shrimp and common name (*Haliotis discus hannai*) against *Vibrio vulnificus*, *Vibrio rotiferianus*, *Vibrio campbellii* and *Vibrio parahaemolyticus* respectively (Zheng and Wang 2017; Gao et al. 2018; Du et al. 2019). An increase in disease resistance was also detected in Pacific white shrimp fed *Lactobacillus* (Zuo et al. 2019). Likewise, supplementation of *Lactococcus* spp. led to the improvement disease resistance of common carp against *A. hydrophila* (Feng et al. 2019); Nile tilapia against *Staphylococcus aureus* (Abdelfatah and Mahboub 2018); olive flounder against streptococcosis (Nguyen et al. 2017) and hump grouper (*Cromileptes altivelis*) against *Vibrio harveyi* (Sun et al. 2018). Similar result was also revealed in rockfish (*Sebastes schlegeli*) fed *P. acidilactici* against *Edwardsiella tarda* (Rahimnejad et al. 2018) and rainbow trout fed *Ent. faecalis* against *Lactococcus garvieae* (Baños et al. 2019). Interestingly, the mixture of LAB together or with other probiotics and immunostimulants resulted in higher disease resistance against bacteria and virus. Combination of *Lb. plantarum* and *Lac. lactis* led to increase disease resistance of Pacific white shrimp against *V. parahaemolyticus* (Chomwong et al. 2018). Likewise, significantly improve disease resistance was recorded in Pacific white shrimp, narrow clawed crayfish and mud crab fed *Enterococcus* spp. and *Pediococcus* spp., or LAB with *B. subtilis*, and yeast against *A. hydrophila* and *V. parahaemolyticus* respectively (Cui et al. 2017; Safari et al. 2017; Wang et al. 2019; Yang et al. 2019). Multiple application of LAB or LAB with other probiotics and immunostimulants also improve disease resistance of many finfish species. Dietary administration of *Lb. plantarum* with *B. velezensis*, orange peel derived pectin and corncob-derived xylooligosaccharide or *C. militaris* significantly enhance disease resistance of Nile tilapia against *Streptococcus agalactiae* (Van Doan et al. 2017, 2019, 2020a). Similarly, an elevation of disease resistance was observed in common carp fed β-glucan, mannanoligosaccharide and *Lactobacillus casei* against *A. hydrophila* (Mohammadian et al. 2019b); Asian seabass fed LAB combined with *B. subtilis*, and yeast (Lin et al. 2017); sea



**Table 2** 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 finfish

Species	Isolated from	Doses and duration	Finfish species	Parameters investigated	References
<i>Lactobacillus plantarum</i>	Commercial probiotic	10 <sup>8</sup> CFU per g 4 weeks	<i>Oreochromis niloticus</i>	↑ Growth performance ↓ Death of Pb-exposed	Zhai <i>et al.</i> (2017)
<i>Lb. plantarum</i>	Commercial probiotic	10 <sup>8</sup> CFU per g 4 weeks	<i>O. niloticus</i>	↑ Growth and feed utilization ↓ Death rate and accumulation of AI	Yu <i>et al.</i> (2017)
<i>Lb. plantarum</i>	Isolated from <i>Acipenser persicus</i>	1.2 × 10 <sup>6</sup> , 0.9 × 10 <sup>6</sup> and 0.56 × 10 <sup>6</sup> CFU per g 80 days	<i>Cyprinus carpio</i>	↑ WG, SGR, FCR, PER, resistance against <i>A. hydrophila</i>	Soltani <i>et al.</i> (2017)
<i>Lb. plantarum</i>	Commercial probiotic	50, 100 or 1000 mg kg <sup>-1</sup> 12 weeks	<i>O. niloticus</i>	↑ Growth performance, villus length, amylase, lipase and protease activity	Dawood <i>et al.</i> (2019)
<i>Lb. plantarum</i>	Commercial probiotic	10 <sup>8</sup> CFU per g 56 days	<i>Salmo trutta caspius</i>	↑ Growth performance ↓ FI	Jami <i>et al.</i> (2019)
<i>Lb. plantarum</i>	Commercial probiotic	0, 10, 20 and 50 pp 50 days/0, 2, 4 and 4 pp 70 days	<i>O. niloticus</i>	↑ Growth performance, resistance against <i>Streptococcus agalactiae</i>	Van Nguyen <i>et al.</i> (2019)
<i>Lactobacillus delbrueckii</i>	Commercial probiotic	0, 1 × 10 <sup>5</sup> , 10 <sup>6</sup> , 10 <sup>7</sup> and 1 × 10 <sup>8</sup> CFU per g 8 weeks	<i>C. carpio</i>	↑ FW, WG, FCR, resistance against <i>A. hydrophila</i>	Zhang <i>et al.</i> (2017)
<i>Lb. plantarum</i>	Intestine of <i>Pangasius catfish</i>	10 <sup>8</sup> CFU per g diet 8 weeks	<i>O. niloticus</i>	↑ Growth performance, resistance against <i>S. agalactiae</i>	Van Doan <i>et al.</i> (2019a)
<i>Lb. plantarum</i>	Intestine of <i>Pangasius catfish</i>	10 <sup>8</sup> CFU per g diet 12 weeks	<i>O. niloticus</i>	↑ Growth performance, resistance against <i>S. agalactiae</i>	Van Doan <i>et al.</i> (2020a)
<i>Lb. plantarum</i> and <i>Lb. bulgaricus</i>	Isolated from <i>Tor grypus intestine</i>	5 × 10 <sup>7</sup> CFU per g 75 days	<i>C. carpio</i>	↑ WG, SGR, FCR	Alishahi <i>et al.</i> (2018)
<i>Lactococcus lactis</i>	Isolated from <i>Cyprinus carpio</i>	5 × 10 <sup>8</sup> CFU 8 weeks	<i>C. carpio</i>	↑ Growth performance, resistance to <i>A. hydrophila</i>	Feng <i>et al.</i> (2019)
<i>Lactococcus garvieae</i>	From raw cow mill	10 <sup>7</sup> cells per g 10 days	<i>O. niloticus</i>	↑ Resistance against <i>Staphylococcus aureus</i>	Abdelfatah and Mahboub (2018)
<i>Lac. lactis</i>	Isolated from wild marine fish	10 <sup>8</sup> CFU per ml 8 weeks	<i>Paralichthys olivaceus</i>	↑ SGR, FCR, resistance against streptococcosis	Nguyen <i>et al.</i> (2017)
<i>Lac. lactis</i>	Isolated from olive flounder gut	10 <sup>9</sup> CFU per g 16 weeks	<i>P. olivaceus</i>	↑ FW, final length, SGR, FE	Nguyen <i>et al.</i> (2018)
<i>Pediococcus acidilactici</i>	Commercial probiotic	0.9 × 10 <sup>7</sup> CFU per g 6 weeks	<i>Lates calcarifer</i>	↑ FW, SGR, SR, FI FCR →	Ashouri <i>et al.</i> (2018)
<i>P. acidilactici</i>	Commercial probiotic	6 × 10 <sup>8</sup> CFU per g 60 days	<i>C. carpio</i>	→ PER, SGR, SR ↓ FCR	Hoseinifar <i>et al.</i> (2019)
<i>P. acidilactici</i>	Commercial probiotic	6.3 log CFU per g <sup>1</sup> 8 weeks	<i>Sebastes schlegeli</i>	↑ Growth performance, resistance against <i>Edwardsiella tarda</i>	Rahimnejad <i>et al.</i> (2018)
<i>P. acidilactici</i>	Commercial probiotic	10 <sup>10</sup> CFU per ml 11 days	<i>Acipenser persicus</i>	↑ Resistance against stress → FW, WG, SGR	Taridashti <i>et al.</i> (2017)
<i>Lactococcus lactis</i>	Isolated from <i>Cromileptes altivelis</i> gut	10 <sup>6</sup> , 10 <sup>8</sup> and 10 <sup>10</sup> CFU per g 4 weeks	<i>Cromileptes altivelis</i>	↑ Growth performance, resistance against <i>Vibrio harveyi</i>	Sun <i>et al.</i> (2018)
<i>Enterococcus faecalis</i>	Commercial probiotic	10 <sup>8</sup> CFU per g 30 days	<i>Oncorhynchus mykiss</i>	↑ Growth performance, resistance against <i>L. garvieae</i>	Baños <i>et al.</i> (2019)
<i>β-glucan</i> , mannan oligosaccharide and <i>Lactobacillus casei</i>	Commercial probiotic	5 × 10 <sup>7</sup> CFU per kg 60 days	<i>C. carpio</i>	↑ Growth rate, feed utilization, lipase, amylase, trypsin and protease activities, resistance against <i>A. hydrophila</i>	Mohammadian <i>et al.</i> (2019b)
<i>Ent. faecalis</i>		10 <sup>8</sup> CFU per g	<i>O. mykiss</i>		

(Continued)

**Table 2 (Continued)**

Species	Isolated from	Doses and duration	Finfish species	Parameters investigated	References
<i>Ent. faecium</i>	Commercial probiotic Caspian roach	30 days 10 <sup>6</sup> , 10 <sup>7</sup> and 10 <sup>8</sup> CFU per g 8 weeks	<i>Caspian roach</i>	↑ Growth performance, resistance against <i>L. garvieae</i> ↑ Growth performance, body protein, intestinal digestive enzyme activities, serum total immunoglobulins	Baños <i>et al.</i> (2019) Tarkhani <i>et al.</i> (2020)
$\beta$ -glucan, mannan oligosaccharide and <i>Lb. casei</i>	Commercial probiotic	5 × 10 <sup>7</sup> CFU per kg 60 days	<i>C. carpio</i>	↑ Growth rate, feed utilization, lipase, amylase, trypsin and protease activities, resistance against <i>A. hydrophila</i>	Mohammadian <i>et al.</i> (2019b)
<i>Lactobacillus</i> spp., <i>Ent. faecium</i> , <i>Bacillus subtilis</i> and <i>Saccharomyces cerevisiae</i>	Commercial probiotics	10 <sup>6</sup> , 10 <sup>7</sup> , 10 <sup>8</sup> and 10 <sup>9</sup> CFU per kg 56 days	<i>L. calcarifer</i>	↑ Growth, feed utilization, resistance against <i>A. hydrophila</i>	Lin <i>et al.</i> (2017)
<i>Lb. plantarum</i> and <i>Cordyceps militaris</i>	Intestine of <i>Pangasius</i> catfish	10 <sup>8</sup> CFU per g 8 weeks	<i>O. niloticus</i>	↑ FW, WG, SGR, resistance against <i>S. agalactiae</i> ↓ FCR	Van Doan <i>et al.</i> (2017)
<i>Lb. plantarum</i> and <i>Bacillus velezensis</i>	Isolated from tilapia gut	10 <sup>7</sup> and 10 <sup>8</sup> CFU per g 30 days	<i>O. niloticus</i>	↑ Growth performance, resistance against <i>S. agalactiae</i> ↓ FCR	Van Doan <i>et al.</i> (2018)
<i>Lb. plantarum</i> , <i>Weissella</i> , <i>Lac. lactis</i> and <i>Ent. faecalis</i>	Isolated from marine fish	10 <sup>9</sup> CFU per g 30 days	<i>Apostichopus japonicus</i>	↑ FW, SGR, SR, resistance against <i>V. splendidus</i>	Li <i>et al.</i> (2018)
<i>Lactobacillus rhamnosus</i> and <i>Lac. lactis</i> subsp. <i>lactis</i>	Commercial probiotics	0.5 × 10 <sup>8</sup> and 1 × 10 <sup>8</sup> CFU per g 6 weeks	<i>O. niloticus</i>	↑ Growth, feed utilization, resistance against <i>S. agalactiae</i>	Xia <i>et al.</i> (2018)
<i>Bacillus</i> sp. <i>SJ-10</i> and <i>Lb. plantarum</i>	Commercial probiotics	1 × 10 <sup>8</sup> CFU per g 8 weeks	<i>P. olivaceus</i>	↑ Amylase, trypsin and lipase activity, resistance against <i>S. agalactiae</i> → Length of villi and microvilli	Jang <i>et al.</i> (2019)
<i>Lactobacillus bulgaricus</i> , <i>Lb. acidophilus</i> and <i>Citrobacter</i>	Isolated from <i>Tor grypus</i> and <i>Cyprinus carpio</i>	5 × 10 <sup>7</sup> CFU per g 60 days	<i>O. mykiss</i>	↑ WG, SGR, PER, PER, amylase, trypsin, lipase, alkaline phosphatase, resistance against <i>Lac. garvieae</i> → Protease ↓ FCR	Mohammadian <i>et al.</i> (2018)
<i>Lactobacillus buchneri</i> , <i>Lb. fermentum</i> and <i>S. cerevisiae</i>	Commercial probiotics	10 <sup>7</sup> CFU per g 130 days	<i>O. mykiss</i>	→ WG, FCR, SGR	Vazirzadeh <i>et al.</i> (2019)
<i>Bacillus</i> spp. + <i>Lactobacillus</i> spp. + <i>S. cerevisiae</i>	Commercial probiotics	10 <sup>8</sup> –10 <sup>9</sup> CFU per kg 12 weeks	<i>P. olivaceus</i>	→ Growth performance ↓ Lipid retention	Niu <i>et al.</i> (2019)
<i>Lactobacillus rhamnosus</i> and <i>Jerusalem artichoke</i>	Commercial probiotics	10 <sup>8</sup> CFU per g 30 days	<i>O. niloticus</i>	↑ SGR, WG, resistance against <i>A. veronii</i> ↓ FCR	Sewaka <i>et al.</i> (2019)

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 shellfish. N/A—no information available; ↑—positive effect; ↓—negative effect; →—no effect.

cucumber fed *Lb. plantarum*, *Weissella*, *Lac. lactis* and *Ent. faecalis* (Li *et al.* 2018); Nile tilapia fed LAB or LAB with Jerusalem artichoke (Xia *et al.* 2018; Sewaka *et al.* 2019); olive flounder fed *Bacillus* sp. and *Lb. plantarum* or *Bacillus* spp. with *Lactobacillus* spp., and yeast (Jang *et al.* 2019; Niu *et al.* 2019) and rainbow trout fed LAB with *Citrobacter* or yeast (Vazirzadeh *et al.* 2019; Mohamadian *et al.* 2019a).

### Immune effects of LAB on finfish and shellfish

The immune effects of LAB on finfish have been the most extensively studied. Therefore, only the recent studies, published in 2018 and 2019, regarding the immune functions of LAB on finfish and shellfish are highlighted in this review (Table 3).

#### Finfish

Juvenile common carp were fed for 56 days with a diet mixed with *Lb. acidophilus*, an isolate from chicken manure, in a three differential dosages, 0.2, 0.4 and 0.6% (Adeshina 2018), and all groups significantly increased numbers of immune cells. When challenged with *Pseudomonas aeruginosa* ( $1 \times 10^7$  CFU per ml) or *A. hydrophila* ( $1 \times 10^7$  CFU per ml), the carp survived in a dose-dependent manner: RPS in *P. aeruginosa* challenge survival rates were 42, 68 and 79% respectively; *A. hydrophila* challenge survival rates were 43, 83 and 78% respectively. Common carp were soaked in the water containing *Ent. faecalis* CgM36 ( $10^6$  CFU per ml), a bacteria isolated from carp for 30 min (Mulyani *et al.* 2018). Following 12 days of maintenance, the carp were challenged with *A. hydrophila* ( $10^6$  CFU per ml). The LAB-treated carp showed an increase in their survival rate 4 days postinfection (50%) compared to the control group (35%). Three strains of other carp commensal LAB (CcB7, CcB8, CcB15) were also tested for their immune effects (Shabirah *et al.* 2019). Carp fingerlings were immersed in the LAB-containing water ( $10^6$  CFU per ml) for 24 h, and this process was repeated three times in a 7-day period. The fish were then challenged with *A. hydrophila* ( $10^8$  CFU per ml). The LAB-treated groups demonstrated significantly increased survival rates (CcB7 72%, CcB8 56%, CcB15 83%) compared to that of the control (33%). Common carp were fed carp-isolated *Lac. lactis* strains (Q-8, Q-9 or Z-2) for 8 weeks at a concentration of  $5 \times 10^8$  CFU each per 1 g of feed ( $5 \times 10^8$  CFU LAB per g) (Feng *et al.* 2019). The *Lac. lactis*-fed fish increased gene expression of both proinflammatory (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12), and anti-inflammatory cytokines (IL-10, TGF- $\beta$ ). However, the *Lac. lactis* Z-2-treated group had a decrease in TGF- $\beta$  levels. Smaller juvenile common carp fed *P. acidilactici* MA18/5M-

containing supplementary diet ( $6 \times 10^8$  CFU per g) for 60 days (Hoseinifar *et al.* 2019), revealed increased total immunoglobulin (Ig) concentration, mucous protease activity and skin lysozyme gene expression. The same LAB fed to beluga (*Huso huso*) for 8 weeks at three concentrations ( $10^7$ ,  $10^8$ ,  $10^9$  CFU per g) (Ghiasi *et al.* 2018), revealed significantly increased total serum Ig level, lysozyme activity and respiratory burst activity in a dose-dependent manner. The immune effect of a soil-origin Lactobacillaceae, *Pediococcus pentosaceus* SL001, was studied on grass carp (*Ctenopharyngodon idella*) (Gong *et al.* 2019). When grass carp were fed *P. pentosaceus* SL001 ( $1 \times 10^9$  CFU per g) for 30 days, the gene expression levels of IgM and C3 complement protein were increased in both the liver and spleen. However, the expression levels of lysozyme, IL-1 $\beta$  and IL-8 were varied, whereas challenged with *A. hydrophila*, the *P. pentosaceus*-treated group displayed a significantly decreased mortality rate during the 7 days postinfection (Con: 90%, *Lb. pentosaceus* SL001: 52%).

Nile tilapia fed host-originated probiotics (*Lb. plantarum* N11 ( $10^8$  CFU per g), *B. velezensis* H3.1 ( $10^7$  CFU per g)) for 15 or 30 days (Doan *et al.* 2018), revealed that fish fed the mixture of the two probiotics significantly increased innate immune parameters in both the 15 and 30 days-feeding groups (lysozyme and peroxidase activities, complement phagocytosis and growth performance), compared to the singular formation-treated groups. When challenged with *S. agalactiae* ( $1 \times 10^6$  CFU) at the 30-day feeding time point, the combined form-treated group showed the highest survival rate (relative percent survival, RPS 58.33%). The singular or combined form of *Lb. rhamnosus* JCM1136 and *Lac. lactis* JCM5805 were fed ( $5 \times 10^7$  CFU per g) to the juvenile Nile tilapia for 6 weeks (Xia *et al.* 2018). Fish fed LAB, significantly increased the transcript levels of IFN- $\gamma$  lysozyme, hsp70 and IL-1 $\beta$  in the intestine and liver. However, there were no significant differences between the single and combined form-fed groups. When challenged with *S. agalactiae* WC1535 ( $2 \times 10^3$  CFU), the fish fed *Lac. lactis* survived at the highest level (con. 19%, *Lac. lactis* 59%). Red tilapia (*Oreochromis* spp.) were fed a synbiotic supplementary diet that included Jerusalem artichoke ( $10 \text{ g kg}^{-1}$ ) and dried *Lb. rhamnosus* GG ( $1 \times 10^8$  CFU per g) for 30 days (Sewaka *et al.* 2019). The red tilapia significantly increased mucin-secreting goblet cell numbers, lysosomal activity, alternative complement (ACH50) activity and total Ig concentration. The RPS of the synbiotic-treated fish was  $76.43 \pm 23.24$  when challenged with *A. veronii* ( $10^7$  CFU per fish).

Olive flounder fingerlings fed *Lac. lactis* I2 ( $10^8$  CFU per g) isolated from olive flounder, for 8 weeks (Hasan *et al.* 2018), displayed significantly enhanced innate



**Table 3** Immunological changes resulted from LAB treatment in fish and shellfish

Host	LAB species	Administration routs and doses	Duration	Immune parameters	References
Juvenile common carp ( <i>Cyprinus carpio</i> ) (21.34 ± 1.85 g)	<i>Lb. acidophilus</i> (strain is not mentioned)	0.2, 0.4, 0.6% supplemented to diet	56 days	↑ Survival rate against <i>Pseudomonas aeruginosa</i> (10 <sup>7</sup> CFU per ml) and <i>Aeromonas hydrophila</i> (10 <sup>7</sup> CFU per ml), immune cell number in blood	Adeshina (2018)
Common carp (Weight not mentioned)	<i>Enterococcus faecalis</i> CgM36	Immersion/ 10 <sup>6</sup> CFU per ml	30 min	↑ Survival rate against <i>A. hydrophila</i> (10 <sup>6</sup> CFU per ml)	Mulyani et al. (2018)
Common carp (average 10 cm)	CcB7, CcB8, CcB15 (species not mentioned)	Immersion/ 10 <sup>6</sup> CFU per ml	24 h × 3 times within 7 days	↑ Survival rate against <i>A. hydrophila</i> (10 <sup>8</sup> CFU per ml)	Shabirah et al. (2019)
Common carp (33.07 ± 0.55 g)	<i>Lac. lactis</i> Q-8, Q-9, Z-2	5 × 10 <sup>8</sup> CFU per g diet	8 weeks	↑ Survival rate during the feedings ↑ Pro-inflammatory cytokine expression in serum (TNF-α, IL-1β, IL-6, IL-12), anti-inflammatory cytokine expression in serum (IL-10, TGF-β), except <i>Lac. lactis</i> Z-2 (TGF-β ↓)	Feng et al. (2019)
Juvenile common carp (10.0 ± 2.5 g)	<i>Pediococcus acidilactici</i> MA18/5M	6 × 10 <sup>8</sup> CFU per g diet	60 days	Skin mucus: ↑ Total immunoglobulin (Ig), protease activity, lysozyme gene expression (248.32 ± 10.21 g)	Hoseinifar et al. (2019)
Beluga ( <i>Huso huso</i> ) 10 <sup>7</sup> , 10 <sup>8</sup> , 10 <sup>9</sup> CFU per g diet	8 weeks	Serum: ↑ Immunoglobulin (Ig), lysozyme activity, respiratory burst	Ghiasi et al. (2018)		
Grass carp 10 <sup>9</sup> CFU per g diet	30 days	↑ Survival rate against <i>A. hydrophila</i> (10 <sup>6</sup> CFU per fish), Liver and spleen: ↑ immunoglobulin M (IgM), C3 complement protein	Gong et al. (2019)	( <i>Ctenopharyngodon idella</i> ) (32.1 ± 9 g)	<i>Pediococcus pentosaceus</i> SL001
Nile tilapia ( <i>Oreochromis niloticus</i> ) (~50 g)	<i>Lb. plantarum</i> N11, <i>B. velezensis</i> H3.1	<i>Lb. plantarum</i> N11: 10 <sup>8</sup> CFU per g feeds + <i>B. velezensis</i> H3.1: 10 <sup>7</sup> CFU per g diet	15, 30 days	↑ Survival rate against <i>Streptococcus agalactiae</i> (10 <sup>6</sup> CFU per fish), growth performance Skin mucus: ↑ lysozyme activity, peroxidase activity, Serum: ↑ lysozyme activity, peroxidase activity, complement phagocytosis	Doan et al. (2018)
Juvenile Nile tilapia (0.20 ± 0.05 g)	<i>Lb. rhamnosus</i> JCM1136, <i>Lac. lactis</i> subsp. <i>lactis</i> JCM1136	5 × 10 <sup>7</sup> CFU per g diet	6 weeks	↑ Survival rate against <i>S. agalactiae</i> WC1535 (2 × 10 <sup>3</sup> CFU per fish) Intestine and liver: ↑ Immune-related gene expression (IFN-γ, lysozyme, hsp70, IL-1β)	Xia et al. (2018)
Red tilapia ( <i>Oreochromis</i> )	<i>Lb. rhamnosus</i> GG	10 <sup>8</sup> CFU per g diet	30 days	↑ Survival rate against <i>Aeromonas veronii</i> (10 <sup>7</sup> CFU per fish)	Sewaka et al. (2018)

(Continued)

Table 3 (Continued)

Host	LAB species	Administration routes and doses	Duration	Immune parameters	References
spp.) (14.05 ± 0.42 g)				↑ Mucin-secreting goblet cell number in the intestine Serum: ↑ lysozyme activity ↑, alternative complement (ACH50) activity, total immunoglobulin concentration	
Olive flounder ( <i>Paralichthys olivaceus</i> ) (14 ± 0.5 g)	<i>Lac. lactis</i> I2	10 <sup>8</sup> CFU per g diet	8 weeks	↑ Survival rate against <i>S. iniae</i> (10 <sup>8</sup> CFU per ml) Serum: ↑ respiratory burst, superoxide dismutase activity, lysozyme activity, myeloperoxidase activity, antiprotease activity, pro-inflammatory cytokine mRNA expression (TNF-α, IL-1β, IL-6)	Hasan et al. (2018)
Olive flounder (35 ± 5 g)	<i>Lb. sakei</i> PO11, <i>Lb. plantarum</i> PO23	10 <sup>11</sup> CFU per g diet	42 days	Gill and head kidney: ↑ Immune-related gene expression (IL-1β, TNF-α, MHC-II, IgM, TCR-β)	Feng et al. (2018)
Humpback grouper ( <i>Cromileptes altivelis</i> ) (3.97 ± 0.54 g)	<i>Lac. lactis</i> HNL12	10 <sup>6</sup> , 10 <sup>8</sup> , 10 <sup>10</sup> CFU per g diet	4 weeks	↑ Survival rate against <i>Vibrio harveyi</i> QT520 (10 <sup>5</sup> CFU per fish) ↑ Respiratory burst of head kidney macrophage (HKMs) Serum at 2 weeks: ↑ acid phosphatase activity, lysozyme activity Serum at 4 weeks: diminished immune parameters	Sun et al. (2018)
Juvenile Asian sea bass ( <i>Late calcalifer</i> ) (12.0 ± 0.2 g)	<i>P. acidilactici</i> MA18/5M	0.9 × 10 <sup>7</sup> CFU per g diet	42 days	Serum: ↑ respiratory burst, lysozyme activity, haemolysis activities Mucus: ↑ lysozyme activity	Ashouri et al. (2018)
Juvenile shabout ( <i>Tor grypus</i> ) (45 ± 10 g)	<i>Lb. casei</i> PTCC1608	5 × 10 <sup>7</sup> CFU per g diet	60 days	Blood: ↑ white blood cell number, haemoglobin concentration Head kidney: immune-related gene expression (IL-1β, TNF-α, IL-8)	Mohammadian et al. (2018)
Juvenile Caspian white fish ( <i>Rutilus frisii kutum</i> ) (0.56 ± 0.02 g)	PrimaLac® ( <i>Lb. acidophilus</i> , <i>Lb. casei</i> , <i>E. faecium</i> , <i>B. bifidum</i> ) (strains not mentioned)	1 g LAB mixture per kg diet	45 days	Skin mucus: ↑ lysozyme activity, alkaline phosphatase activity, protease activity	Mirghaed et al. (2018)
Pacific white shrimp ( <i>Litopenaeus vannamei</i> ) (10 ± 2 g)	<i>Lb. plantarum</i> SGLAB01, <i>Lac. lactis</i> SGLAB02	1.5 × 10 <sup>8</sup> CFU per g diet	16 days	↑ Survival rate against <i>Vibrio parahaemolyticus</i> (10 <sup>4</sup> CFU per ml) Haemolymph: ↑ phenoloxidase activity, <i>LvproPO1</i> , <i>LvproPO2</i> gene expression	Chomwong et al. (2018)
Juvenile Pacific white shrimp (1.3 ± 0.07 g)	<i>Lb. bulgaricus</i> (strain is not mentioned)	10 <sup>7</sup> , 10 <sup>9</sup> CFU per g diet	30 days	↑ Survival rate against <i>V. parahaemolyticus</i> PS-017 (10 <sup>7</sup> CFU per ml) Haemolymph: ↑ total haemocyte number, respiratory burst, prophenoloxidase activity	Roomiani et al. (2018)
Juvenile Pacific white shrimp (0.21 ± 0.01 g)	<i>Lb. pentosus</i> BD6, <i>Lb. fermentum</i> LW2, <i>S. cerevisiae</i> P13	Single (10 <sup>6</sup> CFU per g diet) or mixture (10 <sup>4</sup> , 10 <sup>5</sup> , 10 <sup>6</sup> CFU per g diet)	56 days	↑ Survival rate against <i>Vibrio alginolyticus</i> (10 <sup>5</sup> CFU per g shrimp) Haemolymph: ↑ phenoloxidase activity, respiratory burst, lysozyme activity (except single <i>S. cerevisiae</i> P13 group)	Wang et al. (2019)

(Continued)

Table 3 (Continued)

Host	LAB species	Administration routes and doses	Duration	Immune parameters	References
Japanese abalone ( <i>Haliotis discus hannai</i> Ino) (3.52 ± 0.26 g)	<i>Lb. pentosus</i> (strain is not mentioned)	10 <sup>3</sup> , 10 <sup>5</sup> , 10 <sup>7</sup> CFU per g diet	8 weeks	↓ Natural death rates ↑ Survival rate against <i>V. parahaemolyticus</i> (10 <sup>8</sup> CFU per ml) ↑ Hepatopancreatic superoxide dismutase activity, hepatopancreatic catalase activity, lysozyme activity in haemolymph, acid phosphatase activity in haemolymph	Xiaolong <i>et al.</i> (2018)
Juvenile sea cucumber ( <i>Apostichopus japonicus</i> ) (2.72 ± 0.08 g)	<i>Lb. plantarum</i> , <i>Weissella confusa</i> , <i>Lac. lactis</i> , <i>E. faecalis</i> (strains not mentioned)	10 <sup>9</sup> CFU per g diet	30 days	↑ Survival rate against <i>Vibrio splendidus</i> (10 <sup>8</sup> CFU per ml) Body wall: ↑ alkaline phosphatase activity, acid phosphatase activity, lysozyme activity, superoxide dismutase activity	Li <i>et al.</i> (2018)
Crayfish ( <i>Astacus leptodactylus</i> , Eschscholtz) (27.88 ± 0.27 g)	<i>Lb. plantarum</i> KC426951	10 <sup>7</sup> , 10 <sup>8</sup> , 10 <sup>9</sup> CFU per g diet	97 days	Air-exposure challenge: ↑ total haemocyte number in haemolymph, phenoloxidase activity (except 10 <sup>9</sup> CFU per g group), superoxide dismutase activity, catalase activity, lysozyme activity, total plasma protein	Valipour <i>et al.</i> (2019)

Genera abbreviations: *E.*—*Enterococcus*; *Lac.*—*Lactococcus*; *Lb.*—*Lactobacillus*; *P.*—*Pediococcus*; *S.*—*Saccharomyces*; *W.*—*Weissella*; *B.*—*Bifidobacterium*; *P.*—*Pediococcus*. N/A—no information available. ↑—positive effect; ↓—negative effect; →—no effect.

immune parameters: respiratory burst and the activities of superoxide dismutase, serum lysozyme, myeloperoxidase and antiprotease. Furthermore, the LAB-treated fish increased the gene expression of pro-inflammatory cytokines: TNF- $\alpha$ , IL-1 $\beta$  and IL-6. When challenged with *Streptococcus iniae* (10<sup>8</sup> CFU per ml), higher survival (20%) was revealed compared to control fish (0%). Another olive flounder-originated bacteria (*Lactobacillus sakei* PO11, *Lb. plantarum* PO23) were fed (10<sup>11</sup> CFU per g) in a single form to olive flounder for 42 days (Feng *et al.* 2018), and fish fed LAB increased gene expression of immune genes in the gill and head kidney: IL-1 $\beta$ , TNF- $\alpha$ , MHC-II, IgM and TCR- $\beta$ .

*Lactobacillus lactis* HNL12 isolated from humpback grouper (*C. altivelis*) were fed to humpback grouper juvenile at different concentrations (10<sup>6</sup>, 10<sup>8</sup>, 10<sup>10</sup> CFU per g) for 4 weeks (Sun *et al.* 2018), and all *Lac. lactis*-fed groups increased the activities of respiratory burst, serum acid phosphatase and serum lysozyme up to 2 weeks of feeding. However, those innate immune parameters were diminished thereafter for the remainder of the 4-week experimental time period. When challenged with *V. harveyi* QT520 (1 × 10<sup>5</sup> CFU per fish), The RPSs of the 10<sup>6</sup>, 10<sup>8</sup> and 10<sup>10</sup> CFU per g-fed groups were 31, 53 and 50% respectively. Juveniles of Asian sea bass (*Late calcarifer*) were fed *P. acidilactici* MA18/5M (0.9 × 10<sup>7</sup> CFU per g) for 42 days (Ashouri *et al.* 2018). The *P. acidilactici*-

treated group significantly increased innate immune parameters in serum: respiratory burst, lysozyme and haemolysis activities. However, only the lysozyme activity was enhanced in mucosal immune parameters. When shabouth juveniles (*Tor grypus*) were fed autochthonous *Lb. casei* PTCC1608 (5 × 10<sup>7</sup> CFU per g) for 60 days, the fish significantly increased haemoglobin concentration and white blood cell numbers (Mohammadian *et al.* 2018). In addition, gene expressions of IL-1 $\beta$ , TNF- $\alpha$  and IL-8 were also increased in the head kidney. Juvenile Caspian white fish (*Rutilus frisii kutum*) were fed a mixture of *Lb. acidophilus*, *Lb. casei*, *Enterococcus faecium* and *Bifidobacterium bifidum* (PrimaLac<sup>®</sup>, 1 g kg<sup>-1</sup>) for 45 days (Mirghaedi *et al.* 2018). The fish fed PrimaLac<sup>®</sup> increased the enzyme activities of lysozyme, alkaline phosphatase and protease in the skin mucus.

#### Shellfish

A mixture of two autochthonous isolates, *Lb. plantarum* SGLAB01 and *Lac. lactis* SGLAB02, (1 : 1 ratio, 3 × 10<sup>8</sup> CFU per g each) was fed to Pacific white shrimp for 16 days (Chomwong *et al.* 2018), and LAB feeding significantly increased the enzyme activity of phenoloxidase and the gene expression of *LvproPO1* and *LvproPO2*. When immersion-challenged with *V. parahaemolyticus* (1 × 10<sup>4</sup> CFU per ml), cumulative mortalities in the 10 days postinfection were significantly

reduced: *Lb. plantarum* SGLAB01, 50%; *Lac. lactis* SGLAB02, 40%; the mixture, 36.7%); the control 90%. Juvenile white shrimp fed a commensal *Lb. bulgaricus* in two different concentrations ( $10^7$  and  $10^9$  CFU per g) for 30 days (Roomiani *et al.* 2018). The LAB-fed shrimp significantly enhanced total haemocyte numbers, respiratory burst activity and prophenoloxidase activity. In addition, survival rates were increased significantly in a dose-dependent manner; control 33, 53 and 60.00%, respectively, when challenged with *V. parahaemolyticus* PS-017 ( $10^7$  CFU per ml). Three probiotics (*Lb. pentosus* BD6, *Lb. fermentum* LW2 and *S. cerevisiae* P13) were fed to juvenile white shrimp for 56 days in a single ( $10^6$  CFU per g) or mixed formulation at three different concentrations ( $10^4$ ,  $10^5$  and  $10^6$  CFU per g; Wang *et al.* 2019). The shrimp fed with the probiotics in all cohorts increased phenoloxidase and respiratory burst activities. However, enhanced lysozyme activity was only observed in the groups fed LAB in the individual formulation, but not in the P13D group. When challenged with *Vibrio alginolyticus* ( $10^5$  CFU per g shrimp), the shrimp showed an increase in survival rates: *Lb. pentosus* BD6, 59.3%; *Lb. fermentum* LW2, 60%; *S. cerevisiae* P13, 47%; the control, 27%). However, the mixture-fed groups showed no improvement in survival rate.

Japanese abalone (*Haliotis discus hannai* Ino) were fed *Lb. pentosus*, an isolate from abalone, for 8 weeks at various concentrations ( $10^3$ ,  $10^5$  and  $10^7$  CFU per g) (Xiaolong *et al.* 2018). Surprisingly, the natural death rates of the *Lb. pentosus*-fed groups increased in a dose-dependent manner, 2, 4 and 9%, respectively, although the rates were still lower than that of the control (11%). The LAB-fed groups significantly increased innate immune parameters: lysozyme, acid phosphatase, hepatopancreatic superoxide dismutase and catalase activities. When challenged with *V. parahaemolyticus* ( $5 \times 10^8$  CFU per abalone), mortality rates were decreased dose-dependently in 7 days postinfection: the control 100, 70, 55 and 50% respectively.

Four strains of LAB isolated from marine isolates (*Lb. plantarum* (LP), *Weissella confuse* (WC), *Lac. lactis* (LC) and *Ent. faecalis* (EF)) were fed individually ( $10^9$  CFU per g) to juvenile sea cucumber for 30 days (Li *et al.* 2018). All sea cucumber fed LAB (LP, WC, LL or EF) increased innate immune parameters: alkaline phosphatase, acid phosphatase, lysozyme, superoxide dismutase activities. When challenged with *Vibrio splendidus* immersion ( $10^8$  CFU per ml), survival rates in the 10 days postinfection were significantly increased: the control, 48; LP, 67; WC, 63; LL, 65 and EF: 61%. The expression of immune-related genes varied depending on the types of LAB.

Narrow clawed crayfish were fed *Lb. plantarum* KC426951, an isolate from rainbow trout, in various concentrations ( $10^7$ ,  $10^8$  and  $10^9$  CFU per g) for 97 days (Valipour *et al.* 2019), and probiotic administration significantly increased total haemocyte numbers in a dose-dependent manner in response to an air-exposure challenge. Furthermore, the crayfish enhanced innate immune parameters following a post air-exposure challenge for 24 h: phenoloxidase, superoxide dismutase, catalase, lysozyme and total plasma proteins. However, phenoloxidase activity slightly decreased in all *Lb. plantarum* KC426951-fed groups.

Testing the immune effects of the native commensal microbiomes on their own hosts appear to be a current trend in studies. Isolation of probiotics from the commensal microbiota may be a useful approach to enrich the pool of probiotics. Many studies demonstrated beneficial immunological effects when these autochthonous probiotics were administered to the hosts. However, when the native hosts were fed at high concentrations, some studies showed adverse effects. This may be due to the imbalance of the gut microbiota induced by the excessive feeding of autochthons bacteria. This possibility needs further investigation.

### *Bacillus* as probiotics for finfish and shellfish

Genus *Bacillus* is one of the most frequently used probiotic genera in aquaculture, and in the recent review of Soltani *et al.* (2019b) information was presented on the potential of *Bacillus* as promising probiotics by producing bacteriocins, effect on growth performance, the immune system and disease resistance against pathogens in finfish and shellfish aquaculture. In order to avoid duplication, studies reviewed in the aforementioned review are not addressed in this paper.

An updated overview on the use of *Bacillus* as probiotics for finfish and shellfish are presented in Table 4. Under *in vivo* condition, *B. subtilis*, *B. velezensis*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus thuringiensis* and *Bacillus aerius* increased resistance of finfish and shrimp to pathogenic bacteria including *Streptococcus*, *Aeromonas*, *Vibrio*, *Enterococcus* and *Lactococcus* (Meidong *et al.* 2018; Yi *et al.* 2018; Anyanwu & Ariole, 2019; Di *et al.* 2019; Jiang *et al.* 2019; Li *et al.* 2019; Lin *et al.* 2019; Mukherjee *et al.* 2019a; Peng *et al.* 2019; Soltani *et al.* 2019b; Vogeley *et al.* 2019). *Bacillus* species are also a natural resource for screening new quorum quenching bacteria and are commonly regarded as safe bacteria for the use in aquaculture as agents for improving water quality and disease control (Chen *et al.* 2020).

**Table 4** An updated overview on the effect of *Bacillus* on growth performance, immune response and disease resistance in finfish and shellfish

<i>Bacillus</i> species	Isolated from	Doses and duration	Species	Parameters investigated	References
<i>Bacillus</i> spp.	<i>Litopenaeus vannamei</i>	10 <sup>3</sup> –10 <sup>5</sup> CFU per ml	<i>L. vannamei</i>	↑ Survival ↓ FCR	Kewcharoen et al. (2019)
<i>Bacillus</i> spp.	<i>L. vannamei</i>	10 <sup>7</sup> and 10 <sup>9</sup> CFU per kg/35 days	<i>L. vannamei</i>	↑ PHA, expression of <i>proPO</i> , lysozyme, antilipoplysaccharide factor genes → FW, disease resistance against <i>V. parahaemolyticus</i>	Kewcharoen et al. (2019)
<i>Bacillus</i> spp.	Shrimp pond	1.5 × 10 <sup>7</sup> – 1.5 × 10 <sup>9</sup> CFU per ml/4 days	Zebrafish ( <i>Danio rerio</i> )	↑ Disease resistance against <i>V. parahaemolyticus</i>	Peng et al. (2019)
<i>B. aerius</i> B81e	Hybrid catfish	10 <sup>7</sup> CFU per g/ 60 days	Pla-mong ( <i>Pangasius bocourti</i> )	↑ Growth performance, lysozyme, SBA, complement, PHA, RSB, disease resistance against <i>A. hydrophila</i>	Meidong et al. (2018)
<i>B. cereus</i>	Commercial strain	10 <sup>7</sup> , 10 <sup>9</sup> , 10 <sup>11</sup> CFU kg <sup>-1</sup> / 70 days	Pengze curcian carp ( <i>C. auratus</i> )	↑ FW, SGR, ACP, AKP, glutathione peroxidase ↓ Glutathione, CAT, MDA	Yang et al. (2019)
<i>B. licheniformis</i> T-1	Fresh water pond sediment	2.6 × 10 <sup>8</sup> CFU per fish	<i>D. rerio</i>	↑ Disease resistance against <i>A. hydrophila</i>	Chen et al. (2020)
<i>B. licheniformis</i> MTCC 429	Commercial strain	9.35 × 10 <sup>8</sup> CFU per g/90 days	<i>M. rosenbergii</i>	↑ WG, SGR, PER	Sudha et al. (2019)
<i>B. pumilus</i> A97	Golden pompano ( <i>Trachinotus ovatus</i> )	10 <sup>8</sup> CFU per g/ 56 days	<i>T. ovatus</i>	↑ WG, SGR, FCR, nonspecific immune responses, disease resistance against <i>V. ponticus</i>	Liu et al. (2020)
<i>B. subtilis</i>	Dabry sturgeon ( <i>Acipenser dabryanus</i> )	2 × 10 <sup>8</sup> CFU per g/ 56 days	<i>A. dabryanus</i>	↑ TAC, SOD, IgM, lysozyme, disease resistance against <i>A. hydrophila</i> → Growth performance, MDA	Di et al. (2019)
<i>B. subtilis</i>	Grass carp ( <i>C. idellus</i> ) intestine	2.4 × 10 <sup>7</sup> CFU per g/ 42 days	<i>C. idellus</i>	↑ WG, SGR, MDA, TAC, SOD, CAT, glutathione, expression of SOD, CAT and Gpx genes, IL-10 gene ↓ Expression of TNF-α, IL-1β, IL-8 genes	Tang et al. (2019)
<i>B. subtilis</i>	Commercial strain	10 <sup>9</sup> CFU per kg/ 56 days	<i>L. vannamei</i>	↑ FER, FW, SGR, WG	Tsai et al. (2019)
<i>B. subtilis</i> 7K	Hybrid Hulong grouper ( <i>E. fuscoguttatus</i> × <i>E. lanceolatus</i> ) intestine	10 <sup>8</sup> and 10 <sup>10</sup> CFU per g/28–56 days	<i>E. fuscoguttatus</i> × <i>E. lanceolatus</i>	↑ Growth performance, lysozyme, complement, SBA, RSB, SOD, PHA, Mx gene, IFN gene I, IL-1β, IL-8, TNF-a, Singapore	

(Continued)



Table 4 (Continued)

<i>Bacillus</i> species	Isolated from	Doses and duration	Species	Parameters investigated	References
					grouper irodovirus
Zhou <i>et al.</i> (2019) <i>B. subtilis</i> expressing grass carp reovirus VP4 protein	Commercial strain	$2.3 \times 10^{11}$ spores/fish/day/ 56 days	<i>C. idellus</i>	↑ Expression of IL-4/13A, 46 IL-4/13B and CSF1R genes, BAFF, CD4L, MHC-II, CD8, IL-1β, TNF-α, TGF-β), IFN-I, grass carp reovirus VP4	Jiang <i>et al.</i> (2019)
<i>B. subtilis</i> , <i>B. circulans</i>	Wild shrimp ( <i>Farfantepenaeus subtilis</i> ) intestine	$10^6$ CFU per g/ 60 days	<i>L. vannamei</i>	↑ WG, FW, expression of proPO, LGBP, HEM genes, disease resistance against <i>V. parahaemolyticus</i>	Vogeley <i>et al.</i> (2019)
<i>B. thuringiensis</i> G5-8-3T02	Giant tiger prawn ( <i>P. monodon</i> ) intestine	$3 \times 10^5$ CFU per ml/ 2 days	<i>P. monodon</i>	↑ FW, disease resistance against <i>V. mimicus</i>	Anyanwu <i>et al.</i> (2019)
<i>B. velezensis</i> K2	Grouper intestine	$10^7$ CFU per g/ 28 days	<i>E. lanceolatus</i> ♂ × <i>E. fuscoguttatus</i> ♀	↑ Expression of lysozyme, piscidin, IgM and MyD88 genes, ACP, disease resistance against <i>V. harveyi</i> → FW, complement, AKP ↓ Expression of TLR3 and TLR5 genes	Li <i>et al.</i> (2019)
<i>B. velezensis</i> JW	Grass carp ( <i>Carassius auratus</i> ) intestine	$10^7$ , and $10^9$ CFU per g/28 days	<i>C. auratus</i>	↑ ACP, AKP, GP, IFN-γ gene, TNF-α, IL-1, IL-4, IL-10, disease resistance against <i>A. hydrophila</i> ↓ IL-12	Yi <i>et al.</i> (2018)
<i>B. methylotrophicus</i> <i>B. amyloliquefaciens</i> <i>B. licheniformis</i> <i>B. methylotrophicus</i> + <i>B. amyloliquefaciens</i> <i>B. methylotrophicus</i> + <i>B. licheniformis</i> <i>B. amyloliquefaciens</i> + <i>B. licheniformis</i>	Rohu ( <i>Labeo rohita</i> ) intestine	$1 \times 10^7$ cells per g/ 60 days	<i>L. rohita</i>	↑ WG, SGR, FCR, CF, lysozyme, complement, antiprotease, peroxidase, Ig M, PHA, RSB, disease resistance against <i>A. hydrophila</i>	Mukherjee <i>et al.</i> (2019b)

N/A—no information available. ↑—positive effect; ↓—negative effect; →—no effect. FW = final weight, WG = weight gain, FCR = Food conversion ratio, SGR = Specific growth rate, FER = Feed efficiency rate, proPO = Prophenoloxidase, LGBP = lipopolysaccharide- and β-1,3-glucan binding protein, HEM = haemocyanin, N = nitrogen.

It has been demonstrated that use of *Bacillus* probiotics as the bioremediatory tools in the rearing water of aquaculture species and soil of aquaculture ponds have been exhibited as a feasible way of improving water quality through removing of toxic gases, for example ammonia, nitrite, nitrate and carbon dioxide that are harmful for aquatic organisms (Kewcharoen and Srisapoom 2019; Soltani *et al.* 2019b). *Bacillus subtilis*, *B. licheniformis*, *B. cereus* and *B. coagulans* are suggested as suitable bioremediatory tools for removing of organic detritus, but may not be naturally present in high

enough concentrations in the aquatic ecosystems, that is water column and sediment (Soltani *et al.* 2019b). *Bacillus subtilis* and *B. licheniformis* are suggested as more suitable candidates for bioremediation of aquaculture rearing water (Soltani *et al.* 2019b). It has been shown that use of *Bacillus* to the rearing water can make a balance between the micro-organisms in the water column or in the pond soil through a bacterial competition with a consequence in the decreasing in load of secondary bacterial pathogens (Kumar *et al.* 2016).

*Bacillus* also provide a suitable condition in the GI tracts of fish and shellfish, by improving digestion and absorption of the nutrients, which in turn improve the animal growth performance (Ghosh *et al.* 2019; Meidong *et al.* 2018; Li *et al.* 2019; Soltani *et al.* 2019b; Mukherjee *et al.* 2019b; Zhou *et al.* 2019; Vogeley *et al.* 2019; Tsai *et al.* 2019). However, further studies on mode of actions are needed. Probiotic bacilli can modulate the gut microbiota by bacterial competition, resulting in inhibition of pathogen adherence and colonization to intestinal mucosa (Meidong *et al.* 2018; Vogeley *et al.* 2019; Kuebutornyea *et al.* 2019; Soltani *et al.* 2019a, 2019b). The modulation of finfish and shellfish innate immune responses, for example phagocytic and lysozyme activity, respiratory burst, antiprotease and peroxidase, superoxide dismutase and myeloperoxidase by *Bacillus* have been demonstrated (e.g. Yi *et al.* 2018; Zhou *et al.* 2019). For further information see Soltani *et al.* (2019b). Additionally, *Bacillus* probiotics can cause changes in animal cell physiology, for example neutrophil migration, plasma bactericidal activity and increasing of neutrophil adherence ability, that can eventually result in the improving of immune responses, for example increase in complement activity, immunoglobulin production and cell cytotoxicity (Di *et al.* 2019; Soltani *et al.* 2019b; Li *et al.* 2019). These immune-stimulatory effects by *Bacillus* occur in the gut-associated lymphoid tissue of finfish, although the detail mechanisms required further research works.

### Other probiotics

Information on the use of other probiotics in finfish and shellfish aquaculture are less available. However, in a recent review, Ringø (2020) discussed the effects of *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudomonas*, *Pseudoalteromonas*, *Rhodospiridium*, *Roseobacter*, *Streptomyces* and *Vibrio* on growth performance, immune response and disease resistance in shellfish. In order to avoid overlaps with above mention review, we recommend that readers with interest on this topic to have a closer look at the review of Ringø (2020), and the original papers discussed.

### Commercial probiotics in shellfish aquaculture

Information on the use of commercial probiotics in shellfish aquaculture is available (Ringø (2020), and in order to avoid duplication readers with interest on the topic is recommend to have a closer look at the above mention review.

## Conclusions

The importance of probiotic administration, their beneficial health effects has been discussed in several reviews. Falcinelli *et al.* (2018) discussed the effect of probiotic appetite control, glucose and lipid metabolisms. Even though there is numerous information available on the use of probiotics in aquaculture, there is no concrete evidence to conclude that probiotics are better than immunostimulants or vaccines, the beneficial effects upon the host and their environment ensure that probiotics will remain one of the most promising approaches used to control diseases and the subsequent environmental modifiers. In finfish and shellfish, manipulation of GI tract microbiota by probiotics have been revealed *vs.* control or inhibit adhesion and colonization of pathogenic bacteria in the GI tract, improve digestive enzyme activity and growth performance and enhance immune responses of the host against pathogenic infection or physical stress.

The functionality of gut microbiota, depends on the ability of micro-organisms to interact within the GI tract, which benefit the host through influence on inflammation, metabolism, immunity and even behaviour (e.g. Neuman *et al.* 2015; Boulange *et al.* 2016; Ramírez and Romero 2017). When discussing disease resistance, a stable microbiota and its ability to adhere and colonize the intestine is of importance.

In the review of van Doan *et al.* (2020b) devoted to 'host-associated probiotics' in aquaculture, the authors presented the definition; 'bacteria originally isolated from the rearing water or the GI tract of the host to improve growth and health of the host', and revealed benefits of host-associated probiotics to include improved growth performance, feed value, enzymatic contribution to digestion, inhibit adherence and colonization of pathogenic micro-organisms in the GI tract, increase haematological parameters and immune response, and has gained attention within aquaculture. However, *per se* it is not clear, whether host-associated probiotics are more effective than probiotics from other origins, and this merits further research.

In addition to probiotics may also paraprobiotics (cell wall components; Taverniti and Guglielmetti 2011) serve as an alternative to the use of antibiotics in prevention and treatment of infections caused by pathogens. In this regard, it is of interest to notice that both probiotics and paraprobiotics can bind directly pathogenic bacteria, which limits adherence and colonization of the pathogen to gut cells.

The administration of autochthonous probiotics demonstrated to be beneficial on the immune response of both the finfish and shellfish. However, the high administration levels may be unfavourable to the host due to the

ecological imbalance of the gut microbiota. In addition it is known that the microbiota and microbiota derived products influence the mucosal and systemic immune system in finfish and shellfish, however, the topic merits further investigations.

The sporulation capacity of *Bacillus* gives them advantage due to their heat-tolerance and longer shelf-life in various environmental conditions compared to other probiotics, for example *Lactobacillus* spp. Production of digestive and antioxidant enzymes, and immune gene expression have revealed that probiotic *Bacillus* increase growth and resistance of fish and shellfish to pathogenic microbes.

Most probiotics studies *per se* have focus on different strains of LAB and *Bacillus*, however, the results of comparably limited studies on other probiotics revealed their potential to improve growth performance, physiological responses and disease resistance of different finfish and shellfish species. It seems that these probiotics merits future research. In addition, the importance of water quality management and available reports regarding probiotics bacteria with the ability to improve water quality highlight the importance of these probiotics in aquaculture. Interestingly, some probiotics bacteria which has not received much attention compared to LAB (e.g. *Streptomyces* sp.) are capable of producing chitinase which can resolve the issue of high levels of chitin in insect meal-based diet.

Compared to information available on the use of probiotics in endothermic animals, less information is available in aquatic animals, and several questions needs to be addressed. (i) Bidirectional signalling between the gut, its microbiome and the brain, and how can probiotics beneficially affect this interplay. (ii) How probiotics can improve behavioural—and GI disorder. (iii) Degradation of toxic organic compounds and production of bioactive compounds. Can probiotics degrade antinutritional factors like soybean  $\beta$ -conglycinin and soyasaponins? (iv) Use of bacteriocinogenic LAB strains. (v) Use of probiotics displaying antiviral effect, and evaluate the interactions between probiotics and viral infection. (vi) It is established that the gut microbiota plays a pivotal role in regulating host metabolism, but the effect of probiotic on metabolism of aquatic organisms' merits investigations. (vii) Adherence and colonization, is true colonization possible? VIII) Continuous *vs* pulse administration. (ix) Use of parabiotics *vs* probiotics. If we can clarify these questions, this will hopefully bring us a great step forward to clarify the role of probiotics in aquaculture.

### Conflict of Interest

All authors declare that they have no conflict of interest.

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