Chapter 8

Use of Probiotic Bacteria against Bacterial and Viral Infections in Shellfish and Fish Aquaculture

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Additional information is available at the end of the chapter

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1. Introduction

The term "probiotic" was firstly used to denominate microorganisms that have effects on other microorganisms [1]. Etymologically, the term "probiotic" was originated from the Latin word "pro" which means "for" and the Greek word "bios" which means "life". The best known definition for probiotics was developed by the Food and Agriculture Organization (FAO), that defined them as live microorganisms which when administered in adequate amounts confer a health benefit on the host [2]. According to this description, the potential benefits are varied, and if probiotics were administered to shellfish or fish under intensive culture they could improve their production. It is known that virus and bacterial diseases/infections are one of the most important problems in aquaculture production at present. Probiotics can provide some solutions to this problem through different mechanisms or properties such as the production of inhibitory compounds such as bacteriocins, competition for adhesion sites with opportunistic or pathogen microorganisms, competition for nutrients with other bacteria or an improvement of the immune status (e.g. increase of production of immunoglobulins, acid phosphatase, antimicrobial peptides, improvement of cellular activities, etc.) [3-10]. Several reviews have already documented the benefits of probiotics in shellfish and fish but they mainly focused on their effects in the immune response. Thus, hypothetical and desired results of administering probiotics to shellfish or fish in culture will be improving their antiviral and antibacterial defences, which is the focus of the present review. Firstly, a brief description of probiotics is included, and then a review of the main used probiotics against pathogenic virus and bacteria for shellfish and finally, the same for fish. The novelty of this review is based on the shared ability of probiotics to control both viral and bacterial diseases in shellfish and fish often share, which could be the basis for sustainable aquaculture.

2. Probiotic bacteria

There is a great diversity of tested probiotic bacteria, but only few of them have become in commercial probiotics (Table 1). Thus, further studies are mandatory to expand the use of laboratory described microorganisms with probiotic effects to the commercial level and then be used in the aquaculture industry. The procedure to test and market a probiotic is resumed in Figure 1.

Commercial name	Animal/Human	Reference/Comments
AlCare™	Mammalian	Contains *Bacillus licheniformis*
Alibio®	Fish	[30]
Bactisubtil®	Human	Contains *Bacillus cereus*
Bactocell® PA 10	Fish	[42]
BaoZyme-Aqua	Fish	Contains *Bacillus subtilis*
BGY-35	Fish	[51]
Biogrow®	Mammalian	Contains *Bacillus subtilis* and *B. licheniformis*
Bio-Kult®	Human	Contains *B. subtilis*
BioPlus® 2B	Fish	[73]
Biosporin®	Human	Contains *B. subtilis* and *B. licheniformis*
Biostart®	Fish	Contains a mix of *Bacillus* spp. and *Paenobacillus* sp.
Biovicerin®	Human	Contains *B. cereus*
Bispan®	Human	Contains *Bacillus polyfermenticus*
Cernivet®	Fish	[85]
Domuvar	Human	Contains *Bacillus* spp.
Ecomarine®	Shellfish	
Esporafeed Plus®	Swine	Contains *B. cereus*
Lactobacil	Fish	[45]
Lactopure	Mammalian	Contains *Lactobacillus sporogenes*
Liqualife®	Fish	Contains *Bacillus* spp.
Neoferm BS 10	Mammalian	Contains *Bacillus clausii*
Neolactoflorene	Human	Contains *Lactobacillus* spp. and *Bacillus* spp.
Promarine®	Shellfish	
SanoCare®	Fish	Contains *Bacillus* spp.
SanoGuard®	Fish	Contains *Bacillus* spp.
SanoLife®	Fish	Contains *Bacillus* spp.
Sporolac	Fish	[45]
Sustenex®	Human	Contains *Bacillus coagulans*
Toyocerin®	Fish	[85]

Table 1. List of commercial probiotics, including those for shellfish and fish.

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Probiotics are usually consisting on bacteria but some other microorganisms such as yeast, microalgae or even some fungi. They are mainly used as living cells but some studies have also shown their benefits when supplied as heat-inactivated cells (also known as heat-killed cells), formalin-killed (FKC), freeze-dried, dead cells or cell-free supernatant (CFS). Among the vast number of probiotic species used most information relies on the use of *Bacillus* sp. and *Lactobacillus* sp. Different administration modes have been checked, as bath, intraperitoneal or intramuscular injection and in diet being the bath and diet those preferred for the use in the aquaculture. Moreover, more recently, for oral dietary administration the probiotics can be encapsulated in different ways. Besides that, *Artemia* and rotifers (two main diets larvae in marine larviculture) are usually enriched with probiotics in order to produce benefits in the fish/shellfish larvae.

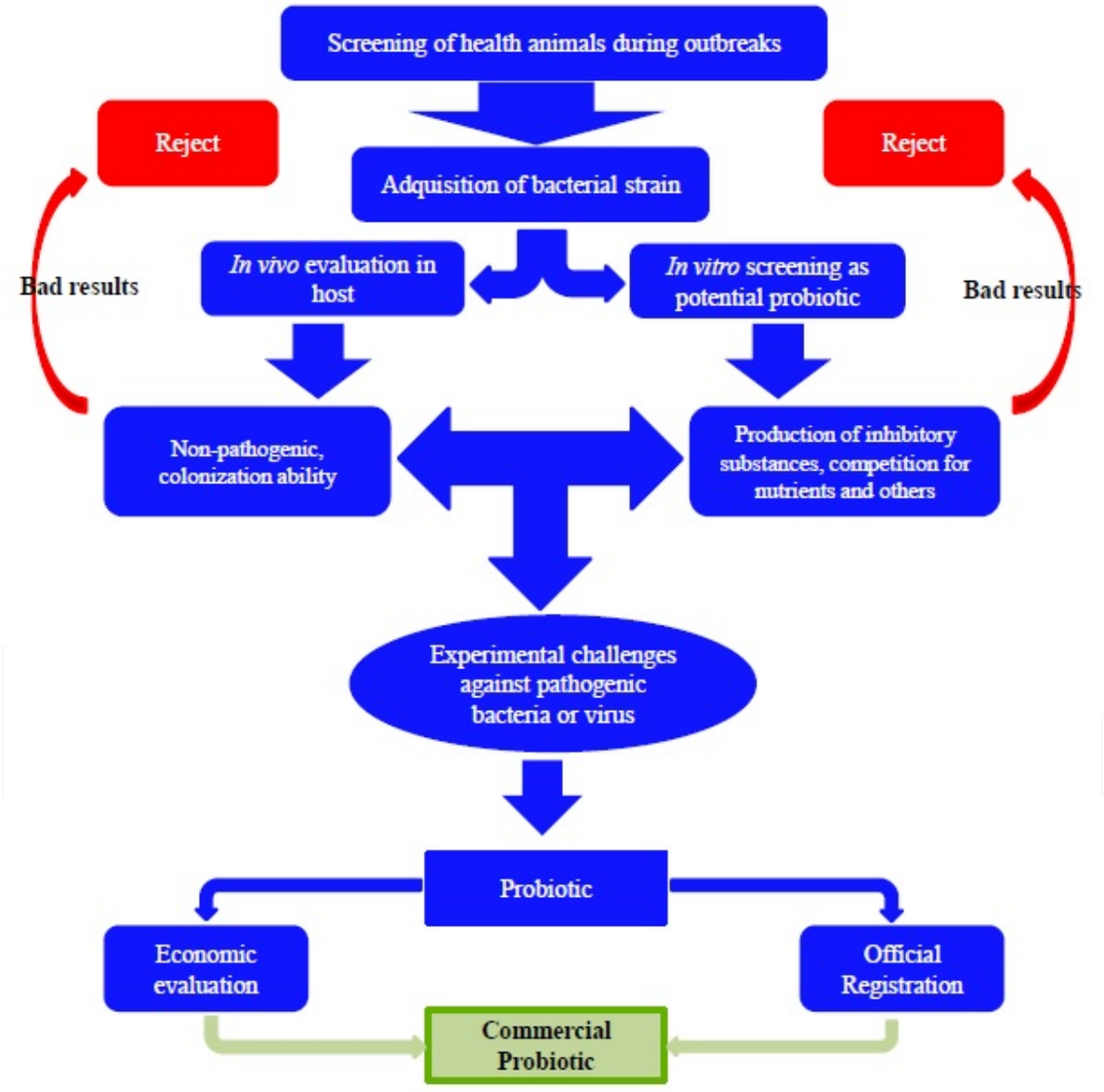

Figure 1. Process for making commercial probiotics.

3. Probiotics against virus in shellfish

Viral infections are one of the most important problems in aquaculture production. In the case of shellfish, probiotics might provide a good preventive solution to this problem since they promote the innate immune response, which is the only one attributed to be responsible for the resistance in these animals.

Mainly seven viral diseases are known in shellfish which are: white spot syndrome virus (WSSV), lymphocystis disease virus (LCDV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), taura syndrome virus (TSV), yellow head disease virus (YHV), infectious myonecrosis virus (IMNV) and *Macrobrachium rosenbergii* nodavirus (MrNV). Unfortunately, all the studies have focused on the potential preventive effects of few probiotics on the pacific white shrimp (*Litopenaus vannamei*) resistance against WSSV. In a single study it was demonstrated that bath treatment of *L. vannamei* specimens with the probiotic *Vibrio alginolyticus* at a dose of 10^5 cfu ml^{-1} showed a higher rate survival against WSSV compared to those non exposed to the probiotic [11]. Interestingly, most of the information comes from studies using dietary administration of the probiotics which results the most desired for aquaculture of shellfish. It has been reported that survival of *L. vannamei* specimens fed supplemented diets containing 10^5 cfu g^{-1} of a mixture formed by lactic acid bacteria (BAL3, BAL7, BC1 and CIB1) failed to protect against WSSV infections [12]. By contrast, dietary administration of 10^{10} cfu g^{-1} of *Bacillus* OJ in *L. vannamei* specimens produced significantly higher survival after challenge by WSSV [13]. It has also been reported that dietary administration of *Pediococcus pentosaceus* and *Staphylococcus hemolyticus* to *L. vannamei* specimens showed a decrease in the prevalence of WSSV, but not IHHNV [14]. Further studies including more shellfish species and virus are necessary in order to find potential solutions for the viral diseases found under their intensive culture.

4. Probiotics against bacteria in shellfish

In the case of bacterial diseases much more studies have focused on the benefits of the use of probiotics for shellfish species. Moreover, and in contrast to the viral pathogens described above, more shellfish species have focused the studies about the use of probiotics. Herein we will summarize the main findings about the potential use of probiotics against bacterial diseases grouped by shellfish species.

A first attempt to describe the probiotic potential of a microorganism comes from *in vitro* studies. Thus, it has been demonstrated that *Pseudoalternomonas* sp. strains DIT09, DIT44 and DIT46 isolated from *Peromytilus purpuratus* showed bacteriostatic anti-*Vibrio parahaemolyticus* activity [15] but their *in vivo* effects have not been tested yet. In a similar way, *Roseobacter* sp. strain BS107 isolated form the scallop (*Pecten maximus*) showed antibacterial activity against several pathogenic *Vibrio* sp. [16] as well as the probiotic *Alteromonas haloplanktis* obtained from *Argopecten purpuratus* larvae specimens [17]. Further preliminary studies of this kind are worthy to be taken in the future and prior to those conducted *in vivo*.

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Several studies have been conducted in bivalves. In the case of Pacific oyster larvae (*Crassostrea gigas*) exposed to 10^5 cfu ml^{-1} of the pathogenic *Vibrio tubiashii* reached a total mortality in just 2 days, whilst in combination with 10^4 cfu ml^{-1} of the probiotic *Aeromonas media* A199 strain the larvae prolonged their viability up to 144 hours indicating its benefits when used by bath [18]. By contrast, *C. virginica* specimens fed supplemented diets containing 10^4 cfu ml^{-1} of *Vibrio* sp. OY15 for three weeks showed no effect in survival ratio after challenge with *Vibrio s*p. M183 [19]. It has been reported [20] that abalone (*Haliotis discus hannai*) specimens fed supplemented diet with 10^9 cfu g^{-1} of *Shewanella colwelliana* WA64 and *Shewanella oyellana* WA65 for four weeks showed a better survival rate (with mortalities of 27%-50% in WA64, and 30%-43% in WA65 compared with 77%-80% in the control group) when infected with *Vibrio harveyi*. In other research with other abalone specie, *Haliotis midae* specimens fed supplemented diet with a mix of three unknown probiotic strains (SY9, SS1 and AY1) at doses of 10^7 cfu ml^{-1} for two weeks showed a better survival ratio (62%) than control group specimens after intra-mantle injection of *Vibrio anguillarum* [21]. Further studies are still needed to broad the use of probiotics in bivalves against bacterial diseases.

Among the shellfish, most of the studies have at this respect focused on shrimps. Thus, western king prawn (*Penaeus latisulcatus*) specimens fed 20×10^5 cfu kg^{-1} diet of *Pseudomonas aeruginosa* and *Pseudomonas synxantha* for eighty-four days and afterwards challenged with *V. harveyi*. *P. aeruginosa*-supplemented diet improved the survival rate of the western king prawns more effectively than *P. synxantha*-supplemented diet, and furthermore, administration of both probiotics in combination resulted in better results than when administering separately [22-23].

Most of the studies administering probiotics have been developed in white shrimp (*Litopenaeus vannamei*) at different development stages. For example, *Bacillus subtilis* E20 administered in the diet at 10^6, 10^7 and 10^8 cfu kg^{-1} increased the survival rates at 13.3%, 16.7% and 20% respectively, after the injection of pathogenic *V. alginolyticus* [24]. In juvenile specimens, commercial white shrimp fed supplemented diet with 10^5 cfu g^{-1} diet of *Bacillus subtilis* UTM126 achieved a mortality of 18% against pathogenic infection of vibrios (including *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus*) while the control group mortality exceeded of 50% [25]. In other research, juvenile specimens fed supplemented diets containing *V. alginolyticus* UTM 102, *B. subtilis* UTM 126, *Roseobacter gallaeciensis* SLV03 or *Pseudomonas aestumarina* SLV22, separately, at doses of 10^5 cfu g^{-1} diet for four weeks showed low mortality (between 17%-22%) after immersion with *Vibrio parahaemolyticus* PS-017 compared with the control group (33%) [26]. In adult specimens of *L. vannamei* fed supplemented diet with 3×10^5 cfu of the probiotic *Vibrio gazogenes* per shrimp showed a decrease of mortality after infection with *Vibrio* spp. (including *V. harveyi*, *V. anguillarum* and *V. alginolyticus*) [27]. In addition, the inhibitory effect was also demonstrated in a *in vitro* assay [27]. Other recent work [28] has been carried out with white shrimp fed a supplemented diet containing 10^5 cfu g^{-1} (BM5) and 10^8 cfu g^{-1} (BM8) (two *Bacillus subtilis* strains) for 2 months, and afterwards each shrimp was injected with 10^7 cfu of *Vibrio harveyi*. Results indicate that cumulative mortality of the control group was 63.3%, whereas in the groups fed probiotics were of 20% and 33.3%, for the group fed BM8 or BM5 strains, respectively. Cumulative mortality also decreased in white shrimp fed a supplemented diet with 10^{10} cfu kg^{-1} of *Lactobacillus plantarum* after injection with *V. alginolyticus* [29].

Moreover, the administration of a mixture of *Bacillus* (*B. endophyticus* YC3-b, *B. endophyticus* C2-2 and *B. tequilensis* YC5-2) to the water at doses of 0.1×10^6 cfu ml^{-1} to juvenile specimens resulted in a high survival ratio (33%) compared with the control group (9.5%) after challenge with *V. parahaemolyticus*. However, a commercial probiotic (Alibio) at the same dose that the *Bacillus* mix had no effect in survival ratio compared with the control group in *Litopenaeus vannamei* specimens [30]. *L. vannamei* specimens fed diet supplemented with two potential probiotics (strains C2 and B6) achieved a better survival ratio (44% and 50%) than control group (21%) after infection with *Vibrio harveyi* in stages from Myosis 3 to postlarvae 1 [31]. Strikingly, other microorganisms such as yeast have been also assayed as potential probiotics. Unfortunately, *L. vannamei* specimens fed *Saccharomyces cerevisiae*, *Phaffia rhodozyma* and *Saccharomyces exiguus* showed no significant different in survival ratio after infection with *V. harveyi* compared with control group specimens [32].

Black tiger shrimp (*Penaeus monodon*) has also received much attention. Thus, *P. monodon* specimens exposed to 10^6 cfu ml^{-1} of *B. subtilis* BT23 for 5 days (long-term treatment) or for 1 hour (short-term treatment), and thereafter challenged with *V. harveyi*, showed a decrease in their cumulative mortality in both groups (32% and 60%, respectively) [33]. In other research, *P. monodon* juvenile specimens fed *Bacillus* sp. S11 at 10^{10} cfu g^{-1} diet for one month and infected with *V. harveyi,* combined with ozone addition, showed a significant increase in the survival ratio (75%) compared with the control group and not fed with probiotics [34]. Also in juvenile specimens fed supplemented diet containing *Lactobacillus acidophilus* 04 at dose of 10^5 cfu g^{-1} for one month showed a higher survival ratio (80%) than the control group (13.3%) after challenged with *Vibrio alginolyticus* [35]. In postlarvae specimens, dietary administration of *Paenibacillus* sp. EF012164 and *Bacillus cereus* DQ915582 at doses of 10^4 and 10^5 cfu ml^{-1} caused lower mortality after infection with *Vibrio harveyi* and *Vibrio* spp. (without statistical analysis) [36]. In other work, *Penaeus monodon* postlarvae specimens fed supplemented diet with 10^9 cfu g^{-1} diet of two strains of *Synechocystis* sp. (C51 and C54) separately for twenty days showed significantly better survival after infection with *Vibrio harveyi* MCCB 111 than those fed without probiotics [37]. Also in postlarvae specimens, dietary administration of *Bacillus* sp. P11 at 10^9 cfu g^{-1} caused a high survival ratio (66%) compared with the control group (0%) after 9 days of infection with *Vibrio harveyi* and *Vibrio* spp. [38]. Dietary administration of *Artemia*-encapsulated *Bacillus* sp. S11 showed an increased survival of *Penaeus monodon* when infected with *Vibrio harveyi* D331 [39]. Finally, dietary administration to *P. monodon* with 10^3 cfu ml^{-1} of *Pseudomonas* sp. PM11 and *Vibrio fluvialis* PM17 for 45 days did not alter the mortality after challenge with *Vibrio anguillarum* [40]. As it has been widely shown in shellfish and fish the use of low or suboptimal dosages of probiotics have no biological role, and in this case protective effect against pathogens.

Other shrimp species have received little attention. In the Indian white shrimp (*Penaeus indicus*) juvenile specimens fed diets supplemented with *Lactobacillus acidophilus*, *Streptococcus cremoris*, *Lactobacillus bulgaricus* 56 or *L. bulgaricus* 57 at doses of 5×10^6 cfu g^{-1} for 4 weeks and infected with *Vibrio alginolyticus* showed a higher survival rate (56% - 72%) compared with that observed in specimens of the control group (20%) [41]. Similarly, in blue shrimp (*Litopenaeus stylirostris*) specimens fed supplemented diet of 10^7 cfu g^{-1} of *Pediococcus acidilactici* for 4 weeks

and infected with *Vibrio nigripulchritudo* SFn1 showed a mortality level of 25% in the probiotic-treated group while in non-treated group the mortality was of 41.7% [42]. It was also reported that *Penaeus chinensis* postlarvae specimens exposed to *Arthrobacter* XE-7 at dose of 10^6 cfu ml^{-1} and pathogenic *Vibrios* sp. (*Vibrio parahaemolyticus*, *Vibrio anguillarum* and *Vibrio nereis*) showed a significant higher survival ratio than specimens exposed to pathogenic *Vibrios* spp. alone [43].

Marron (*Cherax tenuimanus*) specimens fed five probiotics (*Bacillus* sp. AQ2, *Bacillus mycoides* A10, *Shewanella* sp. A12, *Bacillus subtilis* PM3 and *Bacillus* sp. PM4) separately showed no significant differences in survival rate. However, the total haemocyte count was significantly higher in all probiotic-treated groups compared with the control group after injection with 2×10^8 cfu ml^{-1} of *Vibrio mimicus* [44].

Overall, studies have shown that probiotics are good alternative to protect shellfish against pathogenic bacteria, namely against *Vibrio* sp. pathogens, the most important in the culture of shellfish. However, further studies are necessary to broad the probiotic candidates and the shellfish species prior they are applied to aquaculture from a practical point of view. Moreover, the mechanisms behind this protection are generally ignored and deserve deeper evaluation.

5. Probiotics against virus in fish

Viral diseases are major problems in fish farming since there is a lack of suitable antiviral agents and a very limited number of effective vaccines. Moreover, there are few studies about the effects of probiotics against viral infections in fish. Olive flounder (*Paralychthys olivaceus*) and grouper (*Epinephelus coioides*) are the two main species which have been studied. Olive flounder specimens fed 2.4×10^8 cfu g^{-1} of Lactobacil and/or Sporolac (commercial acid lactic bacteria) were infected with lymphocystis disease virus (LCDV) [45]. Lowest mortality rate was seen in groups fed Lactobacil (30%) or Lactobacil and Sporolac (25%) supplemented diets followed by groups receiving Sporolac alone (45%) compared to those groups fed without probiotics that showed a mortality of 80%. Evaluating the disease resistance of grouper through probiotics against virus infection, a recent study has demonstrated that specimens fed a supplemented diet with 10^8 cfu g^{-1} of *B. subtilis* E20 for 28 days showed a survival rate of 50% higher than the control group for seven days post-infection with iridovirus [46]. In another study, grouper specimens fed a diet containing *L. plantarum* at 10^8 cfu kg^{-1} and challenged with an iridovirus showed an increase in the survival of 36.7% compared to the survival rate in control group [47]. Similar results were obtained when grouper specimens were fed *S. cerevisiae* supplemented diet (5.3×10^7 cfu kg^{-1} for four weeks) and afterwards infected with a grouper iridovirus (GIV). Specimens of treated group showed a higher survival ratio (43.3%) than specimens in the control group (16.7%) [48]. Viral pathogens diversity and impact in the actual aquaculture deserves further characterization of the potential benefits of probiotics for economically important cultured fish world-wide.

6. Probiotics against bacteria in fish

By far, the effects of probiotics on fish have received most of the investigations. Among the fish studied, the rainbow trout (*Oncorhynchus mykiss*) has been the most evaluated. Many different probiotic bacteria have been tested and two of the best studied are *Bacillus subtilis* and *Lactobacillus acidophilus,* two lactic acid bacteria which showed *in vitro* inhibition against *Aeromonas hydrophila* [49]. Furthermore, *B. subtilis* avoids the development of *Pseudomonas fluorescens* while *L. acidophilus* had also antimicrobial activity against *Streptococcus iniae*. The information relative to the use of probiotics as a beneficial treatment of fish against bacterial pathogens is described below and summarized (Table 2).

Fish tested	Probiotic	Pathogen	Survival	Cites
Anguilla anguilla	*Enterococcus faecium* SF68 *Bacillus toyoi*	*Edwardsiella tarda 981210L1*	Significant increase for SF68 and no difference for *B. toyoi*	[85]
Anguilla japonica	*Lactobacillus pentosus* PL11	*Edwarsiella tarda*	Significant increase	[87]
Carassius auratus	*Aeromonas hydrophila* A3-51 formalin-inactivated	*Aeromonas salmonicida*	Significant increase	[90]
Carassius auratus *Xiphophorus helleri*	*Bacillus* sp., *Lactobacillus* sp., *Streptococcus faecium*, and *Saccharomyces cerevisiae*	*Pseudomonas fluorescens* 58C	No differences	[89]
Clarias gariepinus	*Lactobacillus acidophilus*	*Staphylococcus xylosus* *Aeromonas hydrophila* gr2 *Streptococcus agalactiae*	Significant increase	[91]
Dicentrarchus labrax	*Vagococcus fluvialis*	*Vibrio anguillarum*	Significant increase	[107]
Epinephelus coioides	*Lactobacillus plantarum*	*Streptococcus* sp.	Significant increase	[47]
	Saccharomyces cerevisiae	*Streptococcus* sp.	Significant increase	[48]
	Bacillus subtilis E20	*Streptococcus* sp.	Significant increase	[46]
Gadus morhua	*Carnobacterium divergens*	*Vibrio anguillarum* *Aeromonas salmonicida*	Significant increase	[57]
Labeo rohita	*Bacillus subtilis*	*Aeromonas hydrophila*	No difference	[96]
	Pseudomonas aeruginosa VSG-2	Aeromonas hydrophila MTC1739	Significant increase	[98]
	Lactobacillus plantarum VSG-3	*Aeromonas hydrophila*	Significant increase	[97]
Miichthys miiuy	*Clostridium butyricum* CB2 as alive and dead cells	*Vibrio anguillarum* *Aeromonas hydrophila*	Significant increase	[94]
Mycteroperca rosacea	*Debariomyces hansenii* CBS-8000339	*Aeromonas hydrophila* AH-315	No difference	[50]

Fish tested	Probiotic	Pathogen	Survival	Cites
Oncorhynchus mykiss	*Clostridium botyricum*	*Vibrio anguillarum*	Significant increase	[95]
	Streptococcus iniae Dan-1 formalin inactivated	*Streptococcus iniae* virulent	Significant increase	[80]
	Pseudomonas fluorescens AH2	*Vibrio anguillarum*	Significant increase	[72]
	Lactobacillus rhamnosus ATCC 53103	*Aeromonas salmonicida* ssp. *salmonicida*	Significant increase	[67]
	Aeromonas hydrophila A3-51 *Vibrio fluvialis* A3-47S *Carnocterium* sp. BA211 Unidentified coccus A1-6	*Aeromonas salmonicida*	Significant increase	[60]
	Aeromonas hydrophila A3-51 *Vibrio fluvialis* A3-47S *Carnocterium* sp. BA211 Unidentified coccus A1-6 formalin-inactivated	*Aeromonas salmonicida*	Significant increase	[62]
	Bacillus subtilis Bacillus licheniformis	*Yersinia ruckeri*	Significant increase	[73]
	Carnobacterium maltaromaticum B26 *Carnobacterium divergens* B33	Yersinia ruckeri Aeromonas salmonicida	Significant increase	[75]
	Lactococcus lactis ssp. *lactis* CFLP100 *Leuconostoc mesenteroides* CLFP196 *Lactobacillus sakei* CLFP201	*Aeromonas salmonicida* ssp. *salmonicida* CLFP501	Significan increase	[63]
	Bacillus sp. JB-1 *Aeromonas sobria* GC2	*Streptoccocus iniae* *Lactococcus garvieae* *Vibrio anguillarum* *Vibrio ordalii* *Aeromonas salmonicida* *Yersinia ruckeri*	Significant increase	[64]
	Bacillus subtilis AB1 as live, sonicated and formalized cells and cell-free supernatant	*Aeromonas* sp.	Significant increase	[82]
	Brochothrix thermophasta BA211 *Aeromonas sobria* GC2	*Aeromonas bestiarum* ORN2	Significant increase	[65]
	Brochothrix thermophasta BA211 *Aeromonas sobria* GC2	*Ichthyophthrius multifiliis*	Significant increase for GC2 and no difference for BA211	[65]
	Leuconostoc mesenteroides CLFP196 Lactobacillus plantarum CLFP238	*Lactococcus garvieae*	Significant increase	[68]

Fish tested	Probiotic	Pathogen	Survival	Cites
	Enterobacter cloacae *Bacillus mojavensis*	*Yersinia ruckeri*	Significant increase	[74]
	Kocuria SM1	*Vibrio anguillarum*	Significant increase	[69-71]
	Lactobacillus plantarum CLFP238 *Lactococcus lactis* CFLP100 *Leuconostoc mesenteroides* CLFP196	*Lactococcus garvieae* CLFP LG1	Significant increase	[66]
	Pseudomonas sp. M174 and M162	*Flavobacterium psychrophilum*	Significant increase	[79]
	Enterococcus faecalis inactivated	*Aeromonas salmonicida*	Significant increase	[81]
Oplegnathus fasciatus	*Lactobacillus sakei* BK19	*Edwarsiella tarda*	No difference	[88]
Oreochromis niloticus	*Lactobacillus acidophilus*, *Bacillus subtilis*, *Clostridium butyricum* and *Saccharomyces cerevisiae*	*Edwardsiella tarda*	Significant increase	[86]
	Bacillus subtilis *Lactobacillus acidophilus*	*Aeromonas hydrophila,* *Pseudomonas fluorescens* *Streptococcus iniae*	Significant increase	[49]
Oreochromis	*Saccharomyces cerevisiae*	*Aeromonas hydrophila* *Pseudomonas fluorescens* *Flavobacterium columnare*	Significant increase	[51]
Paralichthys olivaceus	*Zooshikella* sp. JE-34	*Stretoccocus iniae*	Significant increase	[93]
	Bacillus subtilis *Bacillus pumilus* *Bacillus licheniformis*	*Streptococcus iniae*	Significant increase (except for B. licheniformis)	[92]
Salmo salar	*Vibrio alginolyticus*	*Aeromonas salmonicida* 256/81 *Vibrio anguillarum* VIB256 *Vibrio ordalii* 17K	Significant increase	[52]
	Vibrio alginolyticus	*Yersinia ruckeri* Ex5	No difference	[52]
	Pseudomonas fluorescens AH2	*Aeromonas salmonicida*	No difference	[55]
Salmo trutta	*Lactococcus lactis* ssp. *lactis* CLFP100 *Leuconostoc mesenteroides* CLFP196	*Aeromonas salmonicida*	Significant increase	[83]
Salvelinus fontinalis	S1, S5, S9 and S10	*Flavobacterium columnare*	Significant increase	[84]

Fish tested	Probiotic	Pathogen	Survival	Cites
Scophthalmus maximus	*Roseobacter* sp. strain 27-4	*Vibrio anguillarum*	Significant increase	[108]
	Phaeobacter sp. *Ruegeria* sp.	*Vibrio anguillarum*	Unmeasured	[102]
	Lactobacillus plantarum *Carnobacterium* sp. *Roseobacter* sp.	*Vibrio* sp.	Significant increase	[99]
Solea senegalensis	*Shewanella putrefaciens* Pdp11 *Shewanella baltica* Pdp13	*Photobacterium damselae* ssp. *piscicida*	Significant increase	[104-105]
Sparus aurata	*Shewanella putrefaciens* Pdp11	*Vibrio anguillarum* DC11R2	Significant increase	[103]
	Bacillus subtilis	*Photobacterium damselae* ssp. *piscicida*	No effect	[109]

Table 2. Overview of the effects of probiotics against bacteria in fish.

Few works have evaluated the disease resistance of grouper (*Epinephelus coioides*) through probiotics against the pathogenic *Streptococcus* sp. Thus, dietary treatment of grouper specimens fed *Lactobacillus plantarum* at 10^6 to 10^8 cfu kg^{-1} [47] or 10^8 cfu g^{-1} of *Bacillus subtilis* E20 [46] showed a better survival rate than the control. Moreover, the yeast *Saccharomyces cerevisiae* has shown probiotic effects in the grouper. Feeding with 5.3×10^7 cfu kg^{-1} yeasts four weeks showed a higher survival ratio (56.6%) than the control group (20%) after infection with *Streptococcus* sp. [48].

Leopard grouper (*Mycteroperca rosacea*) specimens fed supplemented diet with 10^6 cfu g^{-1} of *Debaryomyces hansenii* CBS 8339 for five weeks showed an increase in immunoglobulin M (IgM), catalase (CAT) and superoxide dismutase (SOD) after infection with *Aeromonas hydrophila* AH-315 and there was no mortality in any group [50].

Nile tilapia (*Oreochromis niloticus*) fed supplemented diet containing 0.5×10^7 cfu g^{-1} of a mixture of *B. subtilis* and *L. acidophilus*, or 10^7 cfu g^{-1} of each bacteria alone, for two months showed a higher relative level of protection against *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Streptococcus iniae* compared to the control group [49]. The results were even better when fish were fed a commercial probiotic supplemented diet containing *S. cerevisae*. Similar results were also obtained in another two experiments using as a challenge an injection of 2×10^7 cfu ml^{-1} of *P. fluorescens* and fish immersion with 2×10^9 cfu ml^{-1} of *Flavobacterium columnare* [51].

Probiotic bacteria identified as *Vibrio alginolyticus* was inoculated intramuscular or intraperitoneally in atlantic salmon (*Salmo salar*) at doses of 4×10^6 cfu ml^{-1} followed by a bath for ten minutes in a suspension of the same probiotic with 10^8 cfu/ml and seven days later fish were challenged with *Aeromonas salmonicida* 256/81, *Vibrio anguillarum* VIB256, *Vibrio ordalii* 17K or *Yersinia ruckeri* Ex5 [52]. So, this work indicated that application of the probiotic to salmon specimens induced a decrease in mortalities after challenge with *Aeromonas salmonicida* 256/81, and to a lesser extent with *Vibrio anguillarum* VIB256 and *Vibrio ordalii* 17K and does not reduce

mortality with *Yersinia ruckeri* Ex5. In this sense, competition *in vitro* studies will help to elucidate these *in vivo* results. In other work [53] atlantic salmon specimens were fed a supplemented diet with 5×10^8 cells ml^{-1} of the microalgae *Tetraselmis suecica* for 14 days were challenged with fish pathogens. Results showed that use of *T. suecica* as a probiotic supplement was successful in preventing mortalities caused by *Aeromonas hydrophila*, *Aeromonas salmonicida* (strains LL and NG), *Serratia liquefaciens*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri* type I. *Salmo salar* fry specimens which were fed *Lactobacillus plantarum* at dose of 2.5×10^9 cfu g^{-1} and infected with *Aeromonas salmonicida* AL2020 showed a cumulative mortality lower than infected control group [54]. *Pseudomonas fluorescens* AH2 at doses of 10^3-10^5 cfu ml^{-1} in water did not confer protection against *Aeromonas salmonicida* in *Salmo salar* specimens [55]. It has been also reported *in vitro* that the pathogen *Vibrio anguillarum* LFI1243 showed a complete inhibition of growth in presence of *Carnobacterium divergens* strains [56]. This is in accordance with another study showing that *Carnobacterium* sp. isolated from salmon inhibited the growth of both *Vibrio anguillarum* and *Aeromonas salmonicida* in intestinal fish mucus [57]. Interestingly, *Carnobacterium divergens* isolated from *Salmo salar* specimens were also tested as fed probiotics in atlantic cod (*Gadus morhua*) specimens which showed lower mortalities.

The most studied fish specie regarding the potential benefits of probiotics is the rainbow trout (*Oncorhynchus mykiss*). *In vitro* studies have demonstrated the competitive adhesion and production of antagonistic compounds by some lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* CLFP100, *Lactococcus lactis* ssp. *cremoris* CLFP102 and *Lactobacillus curvatus* CLFP150) against fish pathogens, including *Aeromonas salmonicida* ssp. *salmonicida* CLFP 501, *Carnobacterium piscicola* CLFP 601, *Lactococcus garvieae* CLFP LG1, *Vagococcus salmoninarum* CLFP 602, *Yersinia ruckeri* ATCC 29473 and *Vibrio anguillarum* La192 [58]. In another *in vitro* assay authors checked the inhibitory effect of *Carnobacterium* sp. and *Pseudomonas* sp. isolated from gut of rainbow trout against *Vibrio anguillarum*, although there was no correlation with the *in vivo* study since the same probiotic failed to protect them against *Vibrio anguillarum* infection [59]. In rainbow trout specimens fed 10^7 cfu g^{-1} of four putative probiotics (*Aeromonas hydrophila*, *Vibrio fluvialis*, *Carnobacterium* sp. and an unidentified coccus) showed a better survival after intraperitoneal injection of *Aeromonas salmonicida* [60]. However, the same dietary doses of *Carnobacterium inhibens* and *Vibrio alginolyticus* conferred a lower protection against *Aeromonas salmonicida*. These results were correlated with other two studies [52, 61]. In rainbow trout fingerlings, the same four putative probiotics seen previously [60] but administered as formaline-inactivated bacteria showed a lower mortality (4%, 4%, 8% and 0%, respectively) after challenge with *Aeromonas salmonicida* [62] suggesting that the use of dead probiotics has also many benefits for fish. Dietary administration of lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* CLFP 100, *Leuconostoc mesenteroides* CLFP 196, and *Lactobacillus sakei* CLFP 202) at doses of 10^6 cfu g^{-1} for 2 weeks showed a survival rate of 97.8%-100% (versus 65.6% in the control group) when trout specimens were challenged with *Aeromonas salmonicida* ssp. *salmonicida* CLFP 501 [63]. It has been reported that dietary supplementation with *Bacillus* sp. JB-1 and *Aeromonas sobria* GC2 at doses of 2×10^8 and 10^7 cfu g^{-1}, respectively for two weeks led to a higher survival rates in trout after challenge with *Streptococcus iniae* and *Lactococcus garvieae* at doses of 2×10^7 cfu ml^{-1}, and *Vibrio anguillarum*, *Vibrio ordalii*, *Aeromonas salmonicida* and *Yersinia ruckeri* at doses of 3×10^8 cfu ml^{-1} [64]. Thus, survival rates in specimens fed control diets were

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0%-20% whereas in specimens fed probiotic-diets survival rate was 100% in all treatments (with JB-1 and GC2) with all pathogens bacteria except for *Vibrio anguillarum* (87% and 94% respectively) and *Yersinia ruckeri* (94% in GC2 diet). In other study it has been found that dietary administration of *Aeromonas sobria* GC2 at dose of 10^8 cfu g^{-1} and *Brochothrix thermosphasta* BA211 at dose of 10^{10} cfu g^{-1} for two weeks showed a higher survival rate (76% and 88%) than in control group (22%) after intramuscular injection with *Aeromonas bestiarum* ORN2 [65]. In the same experiment, it was demonstrated that GC2 probiotic exerts resistance also against ichthyophthiriasis (caused by the parasite *Ichthyophthirius multifiliis*) however BA211 strain had no effect against this pathogen. An *in vitro* assay tested the inhibitory ability of *Lactobacillus plantarum* strains, *Lactococcus lactis* strains and *Leuconostoc mesenteroides* strains against *Lactococcus garvieae* CLFP LG1 [66]. Other research [67] reported that rainbow trout specimens fed *Lactobacillus rhamnosus* ATCC 53103 at doses of 10^9 and 10^{12} cfu g^{-1} for fifty-one days obtained a reduced mortality (18.9% and 46.3%, respectively) compared with the control group (52.6%) when were infected with *Aeromonas salmonicida* ssp. *salmonicida.* An *in vivo* assay against lactococcosis, dietary administration with lactic acid bacteria (*Leuconostoc mesenteroides* CLFP 196, and *Lactobacillus plantarum* CLFP 238) at doses of 10^6 cfu g^{-1} for four weeks showed a decrease in cumulative mortality (46% and 54%) compared with the control group (78%) in trout specimens after injection with *Lactococcus garvieae* [68]. Following with the development of protection in rainbow trout, specimens were fed a supplemented diet with 10^8 cfu g^{-1} of *Kokuria* SM1 for four weeks and after replacement for control diet they were infected with *Vibrio anguillarum* every week [69]. Interestingly, this relative protection was maximum (87%) just after the end of the probiotic-supplemented diet that was disappearing with the time and was of 71%, 68%, 62% and 36% after two, three, four and five weeks after cessation of probiotic, respectively, representing a sign of gradual loss of effect [70, 71]. In other research, *O. mykiss* specimens exposed to *Pseudomonas fluorescens* AH2 at 10^5 cfu ml^{-1} for 5 days or added *in situ* when challenged with *Vibrio anguillarum* showed a higher survival ratio (56% and 65%, respectively) than specimens exposed to *Vibrio anguillarum* without probiotic (50%) [72]. Dietary administration of BioPlus2B, wich contains two probiotic bacteria (*Bacillus subtilis* and *Bacillus licheniformis*) for four weeks resulted in a better survival ratio (41.7%) compared with Ergosan-diet (8.9%) and control diet (9%) in trout specimens after intraperitoneal injection of *Yersinia ruckeri* [73]. Following with the protection against yersiniosis, dietary administration of 10^8 cfu g^{-1} of *Enterobacter cloacae* and *Bacillus mojavensis* separately for two months achieved a high survival ratio (99.2%) compared with the control group (35%) when infected with *Yersinia ruckeri* [74]. In addition, in other research, dietary administration of 10^7 cfu g^{-1} of *Carnobacterium maltaromaticum* B26 and *Carnobacterium divergens* B33 separately for two weeks conferred protection against *Yersinia ruckeri* with a high survival ratio of 73% and 80% respectively, compared with the control group (13%); and the same probiotics (B26 and B33) also provided protection against *Aeromonas salmonicida* with a survival ratio of 80% in both cases compared with the control group (20%) [75]. *Flavobacterium psychrophilum* is the causative agent of coldwater disease (CWD), also known as rainbow trout fry syndrome (RTFS). Although many types of salmonids are susceptible to RTFS, rainbow trout can be especially impacted due to direct mortality or deformities in surviving specimens leading to economic losses in aquaculture [76, 77]. In order to establish strategies of resistance against

CWD with probiotics, in two studies [78, 79] it was demonstrated the ability of *Pseudomonas* sp. M174 and M162 to inhibit *Flavobacterium psychrophilum in vitro*. In addition, others *in vivo* experiments, rainbow trout specimens fed supplemented diet with *Pseudomonas* sp. M174 (at 4×10^6) and M162 (at doses of 5×10^7-2×10^9 cfu g^{-1}) showed a decrease in cumulative mortality after infection with *Flavobacterium psychrophilum* JIP02/86. Thus, cumulative mortality was 41% in the M174-diet group, 35% in the M162-diet group, and 57% in control groups. In an interesting study, oral vaccines with formalin-killed *Streptococcus iniae* Dan-1 at doses of 3×10^{11} cfu ml^{-1} were inoculated in *Oncorhynchus mykiss* specimens provided them protection against *Streptococcus iniae* virulent at doses of 10^5 cfu ml^{-1} until six months later. The survival ratio was 90% in the treated group and 20% in the control group [80]. As seen in the vast literature the benefits of many probiotics in the culture of rainbow trout is achieved. Furthermore, some papers also demonstrate that probiotics do not need to be alive exclusively. Thus, trout specimens fed supplemented diet with inactivated *Enterococcus faecalis* at dose of 5g kg^{-1} feed showed lower cumulative mortality (40%) than the control group (83%) after challenge with *Aeromonas salmonicida* [81]. Other probiotic forms of *Bacillus subtilis* AB1 such as live cells, sonicated cells, formaline-dead cells and cell-free supernatant were applied as supplement in diets to rainbow trout specimens which achieved a survival of 100% in all forms of probiotic-treatments whereas the survival in control groups was 10-15% after intraperitoneal injection with a pathogenic *Aeromonas* sp. [82].

Other trout species have been slightly evaluated. Thus, brown trout (*Salmo trutta*) specimens fed diets containing lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* CLFP 100 or *Leuconostoc mesenteroides* CLFP 196) at doses of 10^6 cfu g^{-1} for four weeks separately, reduced the cumulative mortality after challenge with *Aeromonas salmonicida* from 37% in the control group to 15% and 9%, respectively. [83]. In the case of brook trout (*Salvelinus fontinalis*), specimens exposed to four potential probiotics (S1, S5, S9 and S10) separately at doses of 10^5 cfu ml^{-1} and one pathogen (*Flavobacterium columnare*) showed a higher survival ratio than specimens exposed to *Flavobacterium columnare* (without probiotics) being S9 the most successful with a cumulative mortality of only 4% [84].

Edwardsiellosis, a bacterial septicaemia caused by the Gram-negative bacterium *Edwardsiella tarda*, is one of the most serious bacterial diseases in cultured eels [85]. So, in a study with European eel (*Anguilla anguilla*), dietary administration with *Enterococcus faecium* SF68 from Cernivet® and *Bacillus toyoi* from Toyocerin® for 2 weeks was followed by challenge with *Edwardsiella tarda* 981210L1. *Bacillus toyoi* did not protected against Edwardsiellosis whilst *Enterococcus faecium* SF68 showed higher rate of survival (73%) compared with the control (45%). In the resistance of Nile tilapia (*Oreochromis niloticus*) against edwardsiellosis, dietary administration of a commercial mix of probiotics that contained *Lactobacillus acidophilus* (1.2×10^8 cfu g^{-1}), *Bacillus subtilis* (1.6×10^7 cfu g^{-1}), *Clostridium butyricum* (2×10^7 cfu g^{-1}) and *Saccharomyces cerevisiae* (1.6×10^7 cfu g^{-1}) for 30 days following infection with *Edwardiella tarda*, provided a cumulative mortality lower than positive control group [86]. Recently, it has been also reported [87] that dietary supplementation of 10^8 cfu g^{-1} of *Lactobacillus pentosus* PL11 in Japanese eel (*Anguilla japonica*) challenged with *Edwardsella tarda* showed an increase in growth performance compared with the control group. In the case of rock bream (*Oplegnathus*

fasciatus) it has been also shown that dietary supplementation with 2.2×10^7 cfu g^{-1} of *Lactobacillus sakei* BK19 and challenged with *Edwardsiella tarda* produced a non-significant decrease in the cumulative mortality [88].

Dietary supplementation of different species of *Bacillus* sp., *Lactobacillus* sp., *Streptococcus faecium* and *Saccharomyces cerevisae* had no effect in survival ratio of ornamental fishes (*Carassius auratus* and *Xiphophorus helleri*) specimens after challenge with *Pseudomonas fluorescens* 58C [89]. However, other study with *Carassius auratus* fed a supplemented diet of formalin-inactivated *Aeromonas hydrophila* A3-51 for twenty days showed a decrease in cumulative mortality compared with the control group after infection with *Aeromonas salmonicida* [90].

African catfish (*Clarias gariepinus*) juvenile specimens were fed a commercial diet supplemented with 3×10^7 cfu g^{-1} of *Lactobacillus acidophilus* for 12 weeks. Then, fish were intraperitoneally injected with 2×10^6 cfu ml^{-1} of *Staphylococcus xylosus*, *Aeromonas hydrophila* gr2 and *Streptococcus agalactiae* separately [91]. At one week post infection, the fish survival rate in control group and in infected groups treated with probiotic diet was 100%, whilst in the groups infected with *Staphylococcus xylosus*, *Aeromonas hydrophila* gr2 and *Streptococcus agalactiae* fed the non-probiotic diet, fish survival recorded was 83.3%, 76.6% and 80.0% respectively.

Olive flounder (*Paralichthys olivaceus*) specimens fed supplemented diet with *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus licheniformis*, separately and at doses 10^{10} cfu g^{-1} for eight weeks showed a higher survival ratio in the case of *Bacillus subtilis* and *Bacillus pumilus* (97.3% and 98.7%, respectively) than specimens in the control group (77.3%) after immersion with *Streptoccocus iniae* [92]. For *Bacillus licheniformis* diet, specimens did not show statistically significant differences in survival ratio (86.7%) compared with the control group (77.3%). In another study, *Paralichthys olivaceus* specimens were fed a diet containing 3.4×10^4 (low dose), 3.5×10^6 (medium dose) and 3.4×10^8 cfu ml^{-1} (high dose) of *Zooshikella* sp. JE-34 and challenged with *Streptococcus iniae* showed their mortality reduced from 85 to htose of the controls 25-40% [93].

Chinese drum (*Miichthys miiuy*) specimens were also fed commercial diet supplemented with 10^8 cfu g^{-1} of *Clostridium botyricum* CB2 in the form of alive cells (CB) or dead cells (D-CB) for 30 days and then challenged with *Vibrio anguillarum* and *Aeromonas hydrophila*, separately. Result showed that survival in chinese drum specimens increased in both groups of probiotic diet compared with the control for both pathogen bacteria [94]. These results are according to other study [95] which demonstrated that dietary administration of *Clostridium botyricum* in rainbow trout (*Oncorhynchus mykiss*) achieved resistance against vibriosis.

Tropical freshwater fish (*Labeo rohita*) specimens were fed a supplemented diet with 0.5×10^7, 10^7 or 1.5×10^7 cfu g^{-1} of *Bacillus subtilis* for two weeks. After challenge by intraperitoneal injection of *Aeromonas hydrophila* O:18, specimens showed increased serum bactericidal activity and granulocyte numbers in probiotic-fed groups compared with the control group [96]. In other work [97] it has been reported that *L. rohita* specimens fed dietary supplementation with 10^6, 10^8 or 10^{10} cfu g^{-1} of *Lactobacillus plantarum* VSG3 for two months showed a higher survival rate (37%, 77% and 63%, respectively) than the control group (14%) after injection of *Aeromonas hydrophila*. In addition, dietary supplementation of 10^7 or 10^9 cfu g^{-1} of *Pseudomonas aerugino-*

sa VSG-2 for two months showed a higher survival rate (66% and 55%, respectively) than in the control group (11%) after injection with *Aeromonas hydrophila* MTCC1739. So, the appropriate administration dose was 10^7 cfu g^{-1} of *Pseudomonas aeruginosa* VSG-2 and 10^8 cfu g^{-1} of *Lactobacillus plantarum* VSG-3 which achieved the better survival rate (66% and 77%, respectively) after challenge with *Aeromonas hydrophila* MTCC1739 [97, 98], demonstrating that probiotics are only effective when administered in adequate doses.

Turbot (*Scophthalmus maximus*) larvae specimens fed rotifers enriched with *Lactobacillus plantarum* and *Carnobacterium* sp. at doses of 10^7-2×10^7 cfu ml^{-1} showed a higher survival ratio (53%) than specimens fed rotifers without probiotics (8%) [99]. Similarly, larvae specimens exposed to *Roseobacter* sp. strain 27-4 at dose of 10^7 cfu ml^{-1} showed a significant decrease in cumulative mortality compared with control larvae specimens. In addition, this *Roseobacter* sp. strain 27-4 was previously tested as antagonist to *Vibrio anguillarum* [100]. When specimens were fed rotifers enriched with *Roseobacter* sp. strain 27-4 and infected with *Vibrio anguillarum*, achieved a decrease in cumulative mortality compared with specimens only infected [101]. It was demonstrated in an *in vitro* assay that *Phaeobacter* sp. and *Ruegeria* sp. are also potential probiotics against *Vibrio anguillarum* in turbot [102].

Gilthead seabream (*Sparus aurata*) specimens were fed a commercial diet supplemented with 10^8 cfu g^{-1} of *Shewanella putrefaciens* (Pdp11) for 15 days and challenged with 3.7×10^7 cfu ml^{-1} of *Vibrio anguillarum* DC11R2a [103]. The mortality of the fish which receiving the diet supplemented with the potential probiotic Pdp11 was 10%, lower than the mortality of the fish that received the control diet (56%).

In other works [104, 105] it has been described the effect of the dietary administration of 10^9 cfu g^{-1} of *Shewanella putrefaciens* (Pdp11) and *Shewanella baltica* (Pdp13) to sole (*Solea senegalensis*) against *Photobacterium damselae* ssp. *piscicida*. The mortality decreased after one and two months with dietary administration of both bacteria compared with the control diet.

In european seabass (*Dicentrarchus labrax*) juvenile specimens, it has been demonstrated that dietary intake of *Artemia* with an acid lactic bacteria (*Lactobacillus delbrueckii* ssp. *delbrueckii*) improved growth of specimens [106]. Dietary administration of 10^9 cfu g^{-1} of *Vagococcus fluvialis* during 20 days in adults resulted in a mortality of 17.3% while in control group (without probiotic) was 30% after exposure to *Vibrio anguillarum* 975-1 [107].

7. Conclusions

Probiotics are usually live microorganisms that administered at adequate doses confer health benefits to the host. In this review we have focused only in those probiotics conferring protection to shellfish and fish species important for the aquaculture against viral and bacterial diseases. Some of the main conclusions are summarized below:

- The most studied probiotics are usually *Bacillus* and *Lactobacillus* species.

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- Dietary administration of probiotics is the preferred for the researchers and farmers. However, bioencapsulation through *Artemia* might be considered a good solution, mainly at larval stages.
- Most of the studies have used live bacteria but other forms such as inactivated, killed, homogenized or even supernatants have also presented good probiotic properties.
- Bacteria are the most known probiotics but other microorganisms such as yeast or microalgae are also suitable and good candidates.
- Although probiotics have probed protection against pathogenic bacteria further evaluation of their potential against virus and parasites is deserved.
- The concentration of the administered probiotic is essential and needs to be optimized for every situation.
- The time of administration is also a very important factor and periods of 2 to 4 weeks of dietary administration seem to be the optimal.
- Only a few potential probiotics tested *in vitro* become in effective probiotics *in vivo* and in commercial probiotics.

Further studies are still necessary to increase our knowledge about the use of probiotics to control bacterial infections in shellfish and fish but much more efforts are needed in the case of viral diseases. This is an important issue for the aquaculture industry that is continuously growing due to the fish and shellfish demand for human consume. Apart from the discovery of new or better probiotic formulations, improvement of their benefits may be helpful. Thus, better and cheaper production methods, administration ways or combination with other preventive/therapeutic measures are welcomed.

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