Chapter 7

Probiotic Bacteria as an Healthy Alternative for Fish Aquaculture

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Additional information is available at the end of the chapter

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Abstract

One of the problems of the aquaculture industry is the presence of pathogenic microorganisms whose proliferation is enhanced when the healthy quality of the culture systems do not meet comply with physical-chemical-biological parameters. In order to improve these problems, less aggressive alternatives to the environment have been sought. This is why probiotic bacteria are proposed as an alternative to the same systems where they will be applied, since they generate greater interest in not presenting a threat to the ecosystem, favor survival, improve the immune system of organisms and have antibacterial properties against pathogenic bacteria. This chapter reviews current research related to the search for marine probiotics for application in the aquaculture industry. Additionally, we deliver results from our work related to the research and application of probiotics. The reported studies demonstrate the positive effects of marine bacteria for their aquaculture application. The evidences found in our work allow us to conclude that larval survival is favored by the application of probiotics in the use of vectors such as rotifers, artemia and biofilms. However, depending on the species of interest, it is necessary to study the market for the biotechnological application of probiotics, to evaluate the feasibility of its production on a larger scale and its commercial feasibility.

Keywords: probiotics, pathogens, fishes, aquaculture, *Seriola lalandi*

1. Introduction

In recent years, the use of antibiotics in aquaculture has been reduced due to the diverse environmental problems that it generates in the ecosystems, as for example, the selection of bacterial strains resistant to antibiotics. The incorporation of antibiotics to the culture species, besides eliminating the pathogenic microbiota, also eliminates bacteria that are beneficial for the same organism. Consequently, the accumulation of these chemicals in the organisms is not safe for human being who is the final consumer. The tendency today is to consume 100% natural foods, in search of a healthier and longer life. Likewise, the care of the environment over time has been regulated in different areas, privileging initiatives that have an environmental vision as a way to promote the care of the planet. In this area, the application of probiotics in fish culture mainly of commercial interest has been investigated for several decades. In this chapter, a bibliographical review of the recent probiotic studies in fish culture and the main results obtained from work on the use of probiotics in *Seriola lalandi* culture are made.

2. Updated definition of probiotics in aquaculture

The word probiotic was first introduced by [1] to describe "substances secreted by one microorganism that stimulate the growth of another." The name probiotic comes from the Greek "pro bios," which means "for life" [2]. Arora & Baldi [3] indicate that to date, there is no legal definition for the term probiotic. However, these authors define it as viable microorganisms with beneficial effect on the host. Akhter et al. indicate that probiotics are microorganisms that are administered orally in a sufficient amount to alter the microbiota (by implantation or colonization) of the specific host and lead to benefits for the host's health [4]. On the other hand, Banerjee et al. define probiotics as living microorganisms that confer beneficial effects to the host (improves immunity, helps digestion, protects against pathogens, improves water quality, and promotes growth and reproduction), and can be used as an alternative to antibiotics [5] (**Figure 1**). Probiotics include Gram-positive, Gram-negative bacteria, and many other organisms such as yeasts, bacteriophage, and single-celled algae [6]. In the field of aquaculture, the concept of probiotic should be

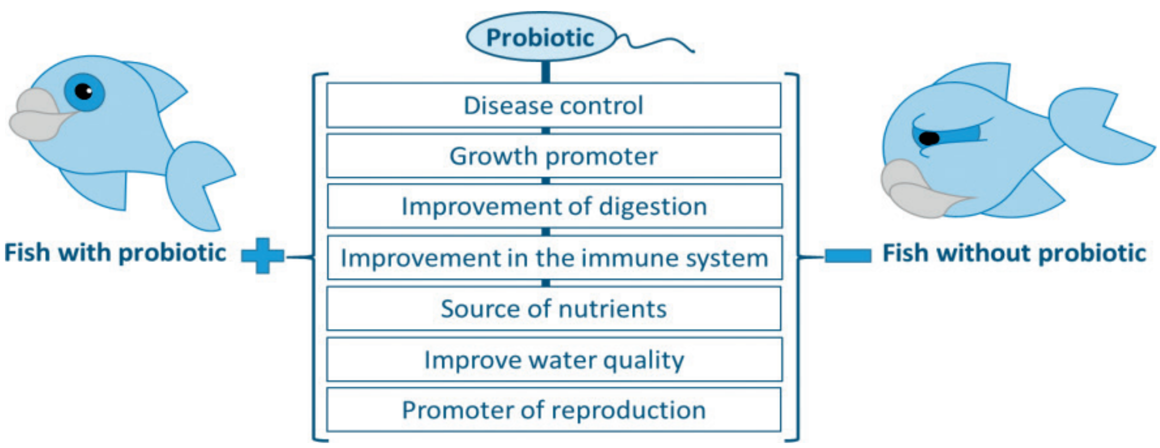

Figure 1. The benefits generated by the dominance of probiotics in confined systems are related to: control in water quality; disease control; promotion of growth; improvement in digestion (enzymes); improvement in immune system; and source of nutrients, among others.

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defined taking into account other influencing factors that differentiate it from terrestrial probiotics. For example, Verschuere et al. extend this definition as "a living microbial complement that has a beneficial effect in the host by modifying the microbial community associated with the host or environment, ensuring a better utilization of the feed or improving the nutritional value, improving the host's response against a disease, or by improving the quality of its environment" [7].

Das et al. suggest that probiotics are a new tool in disease control and improved water quality in the aquaculture industry. Currently, probiotics have become fashionable in the worldwide market as a dietary supplement [8]. The interest in its consumption is related to be within the category of functional/natural foods. Rapid consumer awareness is due to the currently proven therapeutic benefits of probiotics. The benefits associated with probiotics are related to nutrient contribution, to promote survival, to improve the host immune system [4], and to promote growth and/or antibacterial properties against pathogenic bacteria [9]. In addition, probiotics isolated from the same systems where they will be applied, generate greater interest by not presenting a threat to the surrounding ecosystem.

The aquaculture industry is one of the fastest growing food producing sectors in the world, as well as of significant economic importance, expectations of development estimate that much of the food of marine origin and of sweet water in the future will be provided by aquaculture. However, closed crops have threatened industry because of the proliferation of pathogens that until recently were controlled with the addition of antibiotics. The development of bacteria resistant to antibiotics means an enormous risk of transmission from the environment to the human (Pandiyan et al 2013). The development of bacteria resistant to antibiotics means an enormous risk of transmission from the environment to the human [10]. In addition, the use of antibiotics does not discriminate and equally eliminates the beneficial microbiota in the gastrointestinal system of the organisms of interest, as well as, it accumulates in organisms affecting to man as a final consumer [8]. Because of these problems, a global trend has been created that has led to the search for healthy alternatives with the environment to control the pathogens that cause diseases of commercial interest.

The definition of probiotics has evolved over the years, integrating new terms that are related to the new investigations regarding its application in situ. However, the magnitude of the benefit of probiotics will depend on: the concentration of the probiotic; the use of one or a mixture of probiotics of different species; the species and sanitary quality of the host; the stage of development of the host receiving the probiotic supplement (larva, juvenile and/or adult); and the physical-chemical-biological conditions of the environment. Finally, there are many interactions involved that also define the success or failure of probiotic application in culture systems. For this reason, it is fundamental to standardize the protocols, independent for each host species to be treated, since, the success of a probiotic in a specific host, does not guarantee the same beneficial result in another species of host.

3. Influence of diet and water quality on the health fish

Water quality is one of the criteria associated with outbreaks of fish diseases in crops. Therefore, it is essential to maintain water quality parameters that allow the production of disease-free fish [11]. Improving water quality, avoiding the accumulation of organic, nitrogen, ammonia, and nitrite waste are constant concerns in aquaculture crops. High concentrations of these compounds can be extremely damaging and cause massive mortalities [8]. In nature, these toxic substances are transformed into safer forms by the oxidizing bacteria of ammonia (ammonia to nitrite) and oxidizing bacteria of nitrites (nitrite to nitrate) [12].

It has been argued that probiotic bacteria can be used as ecological biocontrol or bioremediation agent for the sustainable development of aquaculture [13–15]. Among the benefits attributed to probiotics are: decreased algae growth, decreased organic load, increased nutrient concentration, increased beneficial bacterial population, inhibition of potential pathogens, and increased concentration of dissolved oxygen [15]. Studies have shown that bacteria of the genus *Bacillus* have been considered as probiotics in water treatment because they have the particularity of converting organic matter into CO_2 [16]. Laloo et al. [17] verified that three isolates of the genus *Bacillus* decreased nitrite, nitrate, and ammonium concentrations in ornamental fish water. This same phenomenon was also observed by Kim et al. [18] with the species *Bacillus subtilis*, *Bacillus cereus,* and *Bacillus licheniformis*, whose effects attributed it to mechanisms such as bioaccumulation, bioassimilation, and nitrification. In addition, it has been proven that the addition of probiotic bacteria reduces the load of pollutants such as heavy metals (Pb, Cd, Hg, Ni, etc.) [19]. Also, the use of *Bacillus* spp. can reduce the incidence of Vibriosis in water [16]. Other probiotic candidates such as *Nitrosomonas* sp. and *Nitrobacter* sp. have been shown to be beneficial in decreasing the pathogenic load in culture ponds [20]. Likewise, the species *Rhodopseudomonas palustris*, *Lactobacillus plantarum*, *Lactobacillus casei,* and *Saccharomyces cerevisiae* have been attributed to probiotic potential in the maintenance of water quality [21].

The application of probiotics for fish culture requires rigorous measures that determine its effectiveness. One of them is related to the abiotic (physical-chemical) or biotic (biological) factors that will stimulate the proliferation and dominance of the probiotic only if the conditions of its surroundings are favorable for this one. The application of probiotic can be done directly to the culture water or mixed with the inoculum of "green water," which is the entrance of microalgae in high concentrations, commonly used in fish culture for food consumption in the initial phase of the larval culture (2 days after hatching). Another pathway of probiotic entry in same fish culture is through live feed that fish receive as rotifers (up to approximately day 19 after hatching) (**Figure 2**), and then the addition of *Artemia* (until about day 25 of culture after of hatching). Another route of entry is through the skin of the fish where probiotics can colonize the surface layer of the skin and then enter through it. Consequently, probiotics after inoculum in culture systems can be found in water, sediment, and organisms of culture (**Figure 3**).

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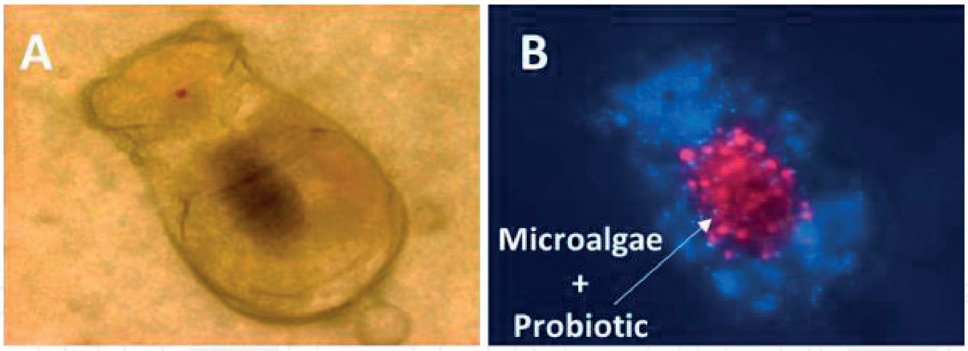


Figure 2. (A) Rotifers (*Brachionus rotundiformis*) in clear field 40×. (B) Rotifers (*B. rotundiformis*) fed with microalgae supplemented with probiotic bacteria stained with DAPI (4′, 6-diamino-2-phenylindole) and visualized by 40× epifluorescence microscopy.

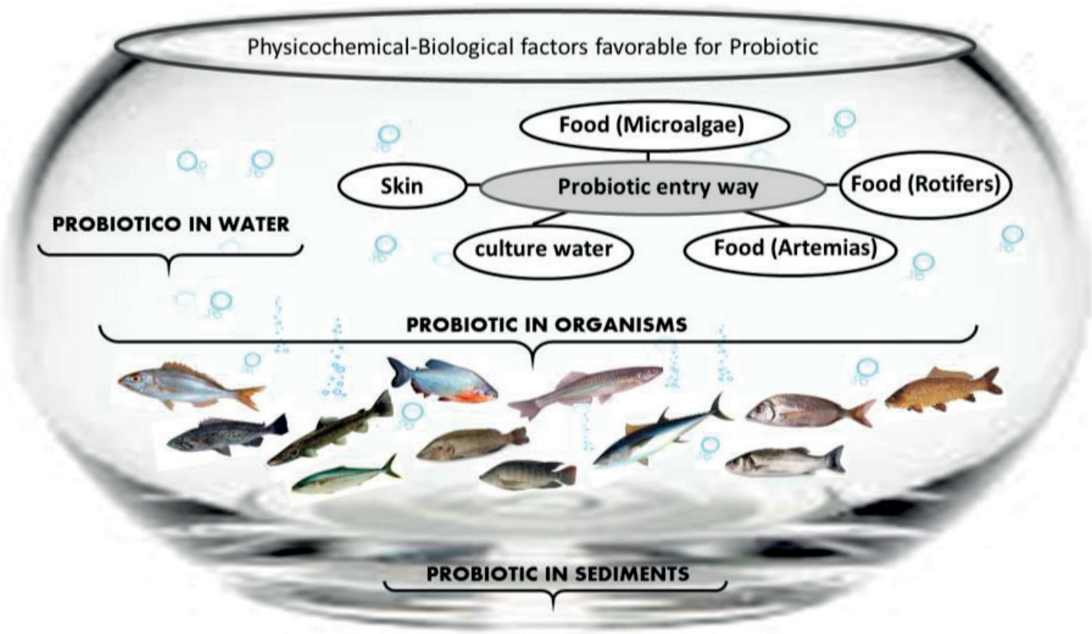


Figure 3. Probiotics in a confined system can be found in water, sediment, and organisms. The success of dominance in the system will depend on its concentration and whether the physical-chemical-biological factors are favorable for its development. Potential routes of entry of probiotics to fish may be more than one among which stand out: culture water; skin; food through microalgae, rotifers and/or *Artemia*.

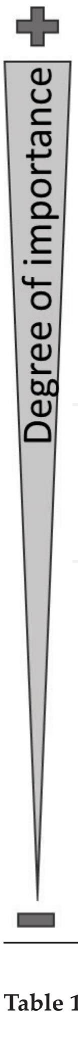
4. Considerations for the selection of probiotics in aquaculture

According to Dawood et al. [22], a probiotic microorganism can meet the needs to develop successful aquaculture because it increases the key factors of yield in growth and disease resistance. Microorganisms intended to be used as probiotics in aquaculture should perform functions that should be considered safe not only for aquatic hosts but also for their environments and humans [23]. According to FAO [24], the probiotic effect on food can have the desired impact only if it contains at least 106–107 live probiotic bacteria per gram or milliliter.

Marine microorganisms have been recognized as potential sources of relatively more stable enzymes than homologous enzymes in terrestrial microorganisms; among them, the salinity, pressure, temperature, and lighting conditions differ. Marine microbial enzymes may enhance host digestion or molecular signals involved in the quorum perception in pathogens for aquaculture disease control [25].

It is essential that the strain selected as probiotic does not pose a risk to the host because of the secretion of antibacterial toxins. The preselection measures are very important and should be taken to evaluate their safety before being categorized as probiotic. In this regard, there are countries that have developed standards for the application of food additives with microorganisms [26]. Some of these norms are related to favoring potential probiotic bacteria isolated from the organism of interest to treat, mainly of the digestive system, since they have a greater capacity of adhesion to gastrointestinal mucus and tissues, compared to the foreign bacteria that are usually transient [27] as well as resistant to low pH.

The mechanism of action of each probiotic in specific is difficult to elucidate, because there are a variety of factors that interact between the probiotic and the surrounding environment. However, **Table 1** highlights essential properties to qualify as a probiotic candidate.



Properties to consider to qualify as a probiotic candidate
– **Absence of hemolysins**, safety for the host [28]. With in vitro techniques such as hemolytic activity and mannitol's ability to use, the biosafety of selected bacterial strains can be checked, as well as in vivo tests (fish supplemented with probiotics) to confirm the non-pathogenic activity of the selected candidates [19]
– **Absence of antibiotic resistant genes** [28]
– **Pathogen antagonist:**
Competitive exclusion: can bind to colon and mucosal cell lines, helping to colonize the intestinal system [28–30]
Ability to produce inhibitory metabolites: such as protease, amylase, cellulose, phytase, chitinase, and lipase [19]. As well as small (peptide)/major (protein) bacteriocins; lysozyme; proteases and hydrogen peroxide) [9, 28, 31]. Or secretion of antimicrobial proteolytic enzymes (aminopeptidase Bs, trypsin-like serine protease, and enzymes reactive against substrates for cathepsin G- and caspase 1-like proteases) [32]
– **Resistant to bile salts and low pH**: one of the routes of introduction of the probiotic is through food [28]
– **Rapid growth and adequate to host/crop temperature** [28]
– **Capacity of adhesion and compete for adhesion sites**: modulates the host's microbiota [9, 31, 33]
– **Improve host immune response** [9, 31, 34–36]. When a pathogen enters the body, the adaptive immune system (B cell and T cell responses) and the complement system are activated [37–39]. Upon attachment to the surface of the mucosa, the probiotic modulates immunity of the mucosal of the fish [34]. The exact mechanism/working path of probiotics in the fish immune system is unclear to date [5]
– **Supplementing essential nutrients,** such as vitamins and enzymes [9, 31].
– **Competing for essential** [9, 31]
– **Regulating neuropeptides involved in signaling pathways to improve reproductive performance and fecundity** [40]
– **Good interaction to apply mix of probiotics:** Variety of probiotic species may exert greater benefit than individual) [15, 41]
– **Viability to storage conditions** [28]

Table 1. Degree of importance of the properties that a probiotic candidate must have.

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5. Application of probiotics in aquaculture of fish

There is currently a variety of research focused on the probiotic search for fish culture. **Table 2** below provides information based on a review of the last 5 years of research on the use of probiotics in fish aquaculture.

Probiotic	Fish tested	Activity	Reference
Bacillus licheniformis (TSB27) *Lactobacillus thuringiensis* *Bacillus Plantarum* *Bacillus subtilis* (B46).	*Sparus aurata* L.	Enhances the immune	[46]
Shewanella putrefaciens Pdp11	*Solea senegalensis*	Modulates the digestive microbiota, an increase in growth	[51]
Bacillus pumilus H2	Fish	Anti-*Vibrio* activity	[47]
Bacillus subtilis WB60	Anguilla japonica	Increased in weight, efficiency in food and protein	[52]
Lac. pentosus BD6, *Lac. fermentum* LW2, *Bacillus subtilis* E20, *Saccharomyces cerevisiae* P13	Asian seabass	Improved either the growth performance or disease resistance of Asian seabass against *A. hydrophila*	[53]
Pseudoalteromonas sp.	*Seriola lalandi*	Increased larval survival	[54]
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Lactobacillus plantarum	*Oreochromis niloticus*	Decreases mortality and improves growth	[56]
Bacillus subtilis *Lactobacillus rhamnosus*	*Labeo rohita*	Increased the value of biochemical components	[57]
Lactobacillus casei	*Keureling* fish (Tor tambra)	Growth performance and feed efficiency increased	[58]
Bacillus sp. MVF1	*Labeo rohita*	Decreased susceptibility to disease	[59]
Bacillus subtilis *Bacillus licheniformis*	Juvenile rainbow trout	Resistance against *A. salmonicida*	[60]
Kocuria SM1 *Rhodococcus* SM2	*Oncorhynchus mykiss*	Produces extracellular enzymes that may have a role in the host digestive processes	[50]
Vibrio lentus	*Dicentrarchus labrax*	Protective effect against Vibriosis caused by *V. harveyi* in sea bass larvae	[61]
Lactobacillus plantarum	Tilapia	Enhanced the growth performance and modulated some hematological parameters.	[45]
Bacillus megaterium PTB 1.4	Catfish	Increased the activity of digestive enzymes and the growth of catfish	[44]
Lactobacillus rhamnosus	*Pagrus major*	Growth-promoting agent and Increases growth	[22]

Probiotic	Fish tested	Activity	Reference
Lactobacillus acidophilus *Bacillus subtilis* *Lactobacillus bulgaricus* *Saccharomyces cerevisiae*	*C. gariepinus*	Increases larval survival	[43]
Bacillus megaterium, *Bacillus polymyxa* *Lactobacillus delbrueckii*	*Oreochromis* sp.	Increased the performance of zootechnical parameters	[42]
Enterococcus casseliflavus	*Oncorhynchus mykiss.*	Capability of improving growth performance and enhancing disease resistance by inmunomodulation	[62]
Pseudoalteromonas sp.	Fish	Inhibitory activity against fish pathogens	[63]
Pseudoalteromonas sp. Cepa MLms gA3	Fish	Inhibitory activity against the pathogen *V. anguillarum*	[48]
Bacillus sp. *Pediococcus* sp.	*Solea senegalensis*	Improvement protection against pathogen outbreaks and	[64]
Lactobacillus plantarum (LP20)	*Seriola dumerili*	Improves immune response and stress	[65]
Lactobacillus mesenteroides SMM69 *Weissella cibaria* P71	*Scophthalmus maximus* L.	Antimicrobial activity against the turbot pathogens *T. maritimum* and *V. splendidus*	[66]
Bacillus subtilis *Bacillus licheniformis* *Bacillus* sp. *Pediococcus* sp.	*Oreochromis* sp.	Resistance to *S. agalactiae*	[67]
Enterococcus faecalis	*Oncorhynchus mykiss*	Favoring growth, stimulation of the immune system and protection of diseases	[68]

Table 2. Bibliographic review of research published in the last 5 years (2013–2017) on the use of probiotics in aquaculture of marine fish.

From this literature review, we can highlight the novel investigations carried out in *Oncorhynchus mykiss, Seriola dumerili, and Sparus aurata* in which it is shown that the probiotics of the genera *Bacillus, Lactobacillus,* and *Enterococcus* have the capacity to influence the immune system. In this regard, the most widely used probiotics in aquaculture are *Bacillus* and *Lactobacillus* because they have better yield in feed conversion, growth rate, weight gain [22, 42, 43], increase in digestive activity [44], increase in the growth performance of the fish [45], immunostimulant [46] and antagonistic activity against *Vibrios* [47]. In addition, according to the literature, it is common to find probiotics of the genus *Pseudoalteromonas* sp. [48, 49]. However, this genus has not yet been explored at the biotechnological level. On the other hand, there are probiotic strains of the genus *Kocuria* and *Rhodococcus*, which have shown a great resistance to the antibiotics and are able to produce extracellular enzymes [50]. This bibliographic review allows us to verify that the study of probiotics for use in fish aquaculture

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is an issue of current interest. The use of specific probiotics will allow controlling organism diseases, water quality of culture, improve survival, and in this way develop a sustainable aquaculture production avoiding the use of antibiotics.

6. Preliminary results of the probiotic search and application in *Seriola lalandi*

In this section, we will introduce the results of research carried out in our laboratory regarding the use of probiotics in *S. lalandi* larvae. This study emerged with the interest of promoting the cultivation of this species in northern Chile, an area not yet developed on an industrial scale.

6.1. Isolation of the probiotic *Pseudoalteromonas* sp. (SLP1-MESO)

The yellowtail *S. lalandi* is a marine species of high commercial demand. However, this species have persistent difficulties with respect to larval survival. Based on the bibliographic background of the benefit of probiotic bacteria in larval fish culture, we isolated and identified bacteria from the gonads microbiota of *S. lalandi* juvenile. The results showed that 42% belong to the genus *Pseudoalteromonas* of the total isolated bacteria (46 strains), nine of which had inhibitory activity against pathogenic bacteria. Of these, *Pseudoalteromonas* sp. (SLP1-MESO) presented inhibitory activity against *Yersinia ruckeri* (35 mm inhibition halo by Dopazo technique) (**Figure 4**) and was the only one that was negative for hemolysis, proteolysis, and lipolysis. These properties make it a good candidate to use as a probiotic in the larval phase of fish culture, which can be incorporated into the fish through the food [63].

6.2. Increased survival of *S. lalandi* using *Pseudoalteromonas* sp. (SLP1-MESO) as probiotic

In order to evaluate the effect of the probiotic potential of *Pseudoalteromonas* sp., isolated from Seriola specimens, this bacterium was added as a probiotic supplement in the culture of *S. lalandi* larvae. For this, larvae of *S. lalandi* cultivated in ponds of 450 lt were fed with rotifers (*B. rotundiformis* and *B. plicatilis*) and *Artemia* sp., which were previously fed with microalgae

Figure 4. From left to right, juvenile *S. lalandi* used for isolation of *Pseudoalteromonas* sp. (SLP1-MESO) image of inhibitory activity by the Dopazo technique observed from the probiotic and the pathogen interaction.

Nannochloropsis gaditana supplemented with the probiotic *Pseudoalteromonas* sp. (SLP1-MESO). The results showed that rotifers and *Artemia* were good vectors of probiotics because *S. lalandi* larvae fed probiotic supplement that had higher survival (**Figure 5**) and length than control at the end of the experiment. These findings show that the probiotic *Pseudoalteromonas* sp. is a good candidate for use in larval cultures of *S. lalandi* [54].

6.3. Cultivation of *S. lalandi* larvae supplemented with probiotics in a mesocosmos system

In order to verify the probiotic effectiveness of *Pseudoalteromonas* sp., on a larger scale, it was evaluated that the survival of *S. lalandi* larvae cultured in a mesocosmos system (50 m^3 Pool) in submerged cages whose cubic structure support (800 lt volume) was composed of PVC pipes and the walls and bottom by mesh (450 μm of Swiss nylon) inoculated *S. lalandi* larvae and fed with *B. rotundiformis* and *B. plicatilis* and *Artemia* sp., supplemented with the probiotic bacterium *Pseudoalteromonas* sp. (SLP1-MESO) and the microalga *N. gaditana*. The survival of the larvae was evaluated until before the change of diet from live food to pellet. The results showed that the addition of the *N. gaditana* microalgae rich in fatty acids and the probiotic bacterium *Pseudoalteromonas* sp. (SLP1-MESO) inoculated in live food of rotifers and *Artemia* improved the survival of *S. lalandi* larvae (**Figure 6**), making it a good dietary alternative to optimize larval survival of this species, being able to be applied to other crops of interest commercial [69].

6.4. Use of biofilm as transfer vector of the probiotic *Pseudoalteromonas* sp. (SLP1-MESO)

The use of fixed biofilms meshes (Nylon Sefar Switzerland, 450 μm) was evaluated as a vector to incorporate specific microalga-probiotic food and as a biological control for the benefit of *S. lalandi* larvae. Biofilms were composed of a mixture of diatoms dominated by *Navicula phyllepta* and bacteria of the family Rhodobacteraceae that were previously isolated from biofilms formed in culture cages of *S. lalandi* larvae. In addition, these specific biofilms were tested with the addition of the probiotic *Pseudoalteromonas* sp. (SLP1-MESO). The meshes with biofilms

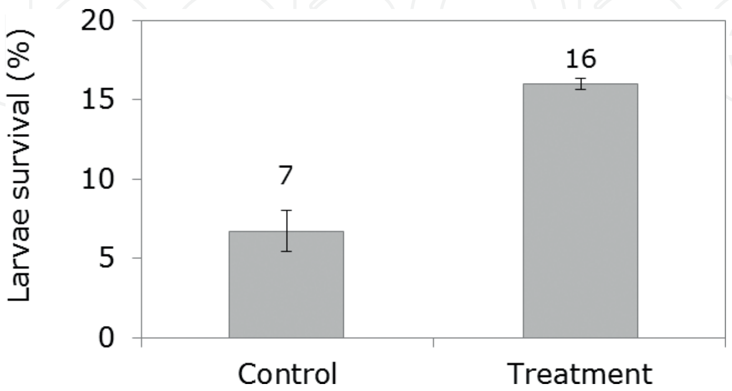

Figure 5. Evaluation of the survival at day of *S. lalandi* larvae fed with probiotics. Supplemented with probiotic bacteria (treatment) and without probiotic bacteria (control). Bars represent ± standard error of the mean. (Figure obtained from Leyton et al. [54]).

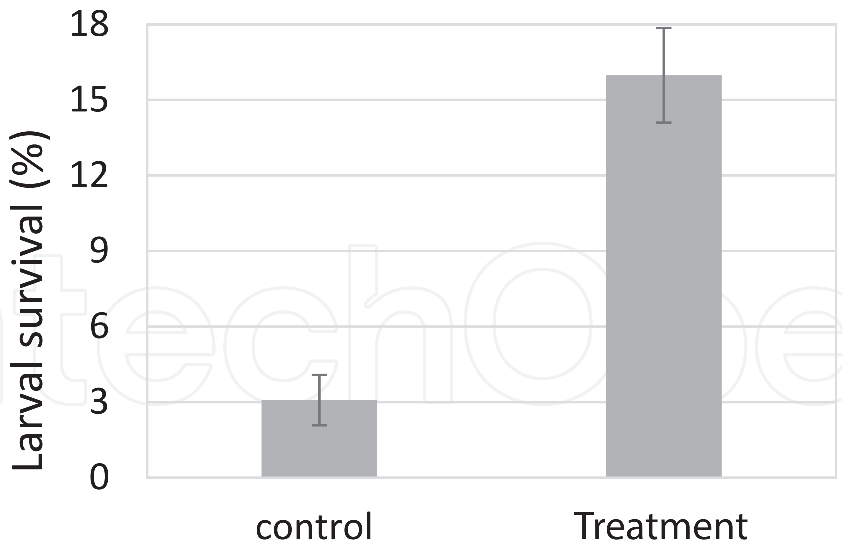

Figure 6. Survival (%) of *S. lalandi* larvae grown in cages in mesocosmos systems. Significant differences were observed between bacteria supplemented with probiotic (treatment) and bacteria without probiotic (control) (t-test = 4.896, $p < 0.05$). Control: *N. gaditana* + *B. rotundiformis* + *B. plicatilis* + *Artemia* sp. + larvae. Treatment: *N. gaditana* + *B. rotundiformis* + *B. plicatilis* + *Artemia* sp. + *Pseudoalteromonas* sp. SLP1 + larvae. Bars represent ± standard error of the mean. (Figure obtained from Plaza et al. [69]).

were immersed in ponds of 200 lt; during 10 days, the consumption and larval survival were evaluated. The results showed that the larvae consumed 70% of the biomass at 72 h in treatment and control without any negative effects on larvae or significant differences. However, a positive survival effect was observed in the biofilms treatments with probiotics obtaining 31% of survival compared to 13% of the control (**Figure 7**). These results demonstrated that this pathway of probiotic entry could be a good alternative for improving the survival of *S. lalandi* larvae [56].

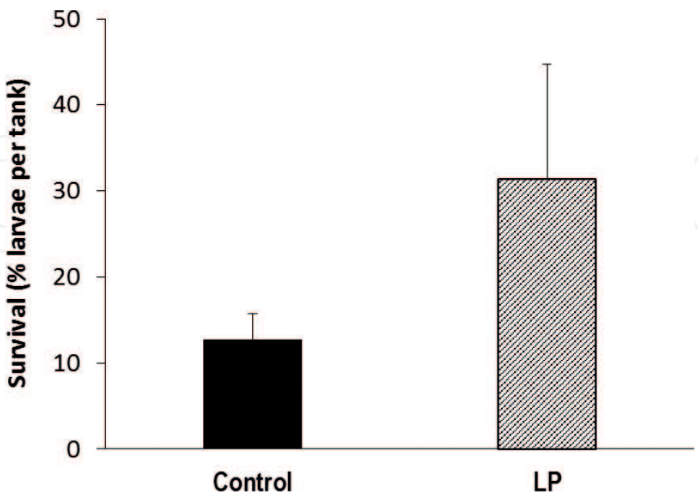

Figure 7. Survival of larvae at the end of the experiment in 200 lt tank. LP: larvae tank treated with probiotics and biofilm. Control: larvae tank with probiotics without biofilm. Data represent the mean and standard deviation of larvae for two replicate tanks for each condition and a triplicate mesh per tank in 400 lt tank and three replicate tanks for each condition and a triplicate mesh per tank in 200 lt tank. (Figure obtained from Mata et al. [55]).

Finally, the analysis of the results obtained in these four research works would indicate that the bacterium *Pseudoalteromonas* sp. (SLP1-MESO) isolated from gonads of healthy juveniles of *S. lalandi*, is a good candidate to be used as a probiotic in the initial larval stages of this species, that is, before the transition from live food to pellet. Our results supported the background of this chapter on the benefits of probiotic bacteria to improve the survival of fish larvae. The different investigations on probiotics in aquaculture have been validating their use to improve the survival of organisms in culture. Probiotic production will be necesary por the future of aquaculture industry.

7. Conclusion and future perspectives

The marine microbial world does not stop surprising us, for its varied potential beneficial to animal health. Based on the literature cited in this chapter, it is evident that the probiotic search for fish application is wide. However, research must be strengthening with new biotechnological processes that allow the mass production and application of probiotics on an industrial scale at an attractive cost. In order to advance in this area and transfer the results of the research from laboratory to the industry, we must overcome some non-minor gaps, such as the legal permit that involves working with living organisms for human consumption. Despite this, it is comforting the increase in worldwide support of respect to the use of probiotics, is becoming a trend in the search for natural solutions to care the environment and to take advantage of what nature offers us.

The authors of this chapter continue to concentrate their research on the application of probiotics in the larval phase of organisms of commercial importance such as fish, molluscs, and currently echinoderms. We have the complete conviction that our specific marine wealth, located in front of the most arid desert in the world, will provide us with the solution to optimize aquaculture in phase larval stages, which will allow to increase the sustainability of aquaculture activity in Chile and South America.

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