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Review

Administration of Probiotics in the Water in Finfish Aquaculture Systems: A Review

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Received: 10 July 2018; Accepted: 20 August 2018; Published: 22 August 2018

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Abstract: Over the last few decades, the contribution of aquaculture to animal protein production has increased enormously, and the sector now provides almost half of the fish and shellfish consumed worldwide, making it a major food producer. Nevertheless, many factors, including infections, pollution, and stress, may result in significant economic losses. The aquaculture industry will not be totally successful without the therapeutic and preventive means to control all these factors. Antibiotics (long used in aquaculture practice) have tended to aggravate the problem by increasing antibiotic resistance. Concomitantly, probiotics have widely been suggested as eco-friendly alternatives to antibiotics. However, the way in which probiotics are applied in aquaculture is a key factor in their favorable performance. The aim of this review was to examine the current state of probiotics administration through the water in finfish aquaculture. The review also attempts to cover the research gaps existing in our knowledge of this administration mode, and to suggest the issues that need to be investigated in greater depth.

Keywords: probiotics; administration method; finfish; favorable performance; aquaculture

1. Introduction

Aquaculture has been accelerating for decades, although outbreaks of a wide variety of infections have resulted in devastating losses economically. In contemporary aquaculture industries, the main focus has been on the use of medicines and chemical additives [1]. Due to the negative effects of chemicals and antibiotics on the environment, followed by the development of mutagenic microbial strains and adversely affected fish health, their application to control disease outbreaks is no longer recommended [2]. Therefore, the application of eco-friendly feed additives, such as microbial supplements, to improve the physiology, growth performance, and immune responses of aquaculture-related species have gained much more attention during recent years [3–5]. Naturally-occurring microorganisms play a key role in aquatic environments, as they can fulfil a wide range of roles, including recycling nutrients, degrading organic matter, and protecting fish against infections [6]. All these roles conduced to use these microorganisms in aquaculture and the development of probiotics. The use of probiotics is one of the alternative approaches to immunoprophylactic control in aquaculture [7,8], and is considered a supplementary strategy or alternative to using vaccines and chemicals [9,10]. The definition of 'probiotic' has been modified over the years. According to the adopted definition of The Food and Agricultural Organization and World Health Organization (FAO/WHO), 'probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [11]. However, according to some authors, 'probiotics applied in aquaculture are microbial cells (live or dead) or constituents of microbial cells that, when added to the water or feed, improve the general health of the host organism via improvements in the microbial balance in the environment'. Overall, probiotics are considered bio-friendly agents that can be administered in aquatic culture environments to control pathogens and enhance feed utilization, survival, and growth rate of farmed species. Furthermore, they do not have any undesirable side effects on treated organisms [12–14]. Whatever the definition, probiotics represent a new era in modern aquaculture, and both commercial and scientific interest in this topic is increasing. Indeed, nowadays, probiotics are commonly used as therapeutic and prophylactic supplements [15–20]. Lactic acid bacteria (LAB) such as some *Lactobacillus* species (e.g., *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus thermophillus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* etc.) are frequently used as probiotics in fish nutrition [16]. The use of LAB has been shown to have the most promising effects on disease resistance, survival, and growth parameters for a wide variety of fish species [8,10,15,21–26]. However, probiotics in aquaculture encompasses a wide range of bacteria (Gram-negative or Gram-positive), yeasts, unicellular algae, and bacteriophages [27,28].

It has been established in different studies that probiotics are able to produce inhibitory substances, enhance immunity, and prevent the colonization of pathogens in the gut [15,24,29–33].

Management systems in aquatic and terrestrial environments are extremely different. One particular example can be the administration of drugs. There are several problems with drug applications in aquatic environments [34]. Several routes for probiotic administration are used in aquaculture systems (Figure 1). Probiotics can be administered as dietary supplements (via live food such as *Artemia* and rotifers or pellet food) or added to the water directly [35,36]. Furthermore, probiotic delivery via injection has also been reported [37]. The use of a suitable administration method contributes to the favorable performance of probiotics, and the knowledge of the action modes, along with suitable administration methods, can play a key role in their application in aquaculture [3].

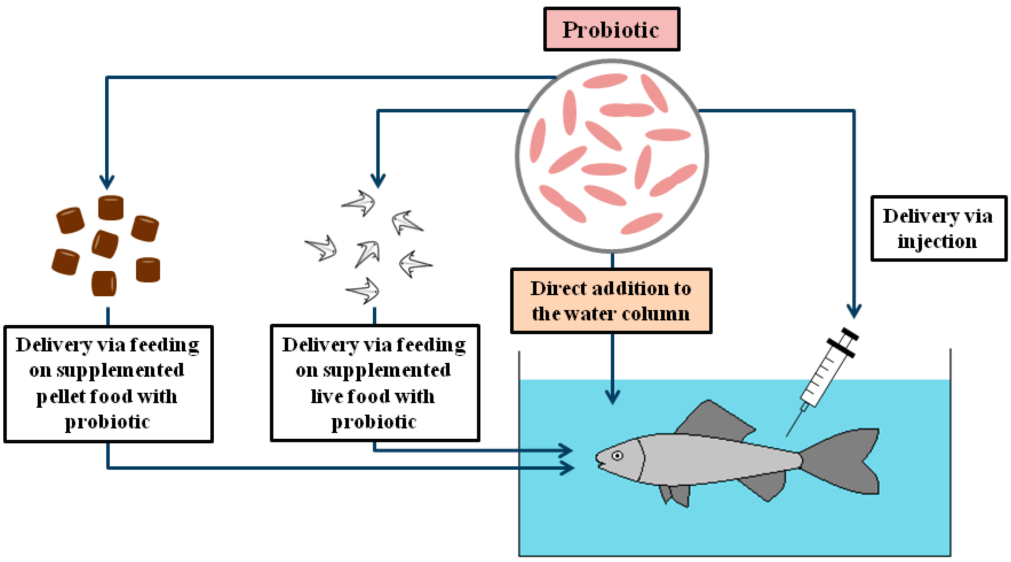

Figure 1. Different routes of probiotics administration in aquatic environment.

To date, most of the studies investigating the properties of probiotics in fish aquaculture have used dietary supplementation, and little attention has been given to possible beneficial effects of direct distribution of probiotics in water. This review summarizes our present knowledge concerning this administration method in finfish aquaculture systems.

2. The Range of Probiotic Administration through Water

Among all the routes of probiotic administration in aquaculture (Figure 1), supplementation of rearing water is the only method which is applicable for all ages of fish. The administration via feeding (dry feed) definitely has limitations during early larval stages due to immature digestive tracts of fish in that stage of development. Furthermore, injection, which is not also applicable for larvae, results in a high level of stress. In contrast, direct addition of probiotics to the rearing water can be applied from the first day of hatching in incubators. The combination of probiotic administration through water and enriched live feed (especially rotifers) has been strongly recommended as the appropriate way to apply probiotics in larviculture. The commercial probiotic (Remus®, Avecom, Ghent, Belgium) directly added to the water containing cod larvae (*Gadus morhua* L.), as well as enriched rotifers upregulated growth-related proteins and downregulated proteins related to stress [38]. According to the results, 70% of the microbial population in the intestine of cod larvae was composed of *L. plantarum* when the rearing water was inoculated with this probiotic bacterium [24]. Overall, administration via rearing water has been the most beneficial administration mode of probiotics in cod larviculture [18].

3. Benefits of Probiotic Administration through Water

According to some findings, a high level of incorporation of probiotic bacteria into treated aquatic organisms (especially in marine environments) has been observed when probiotics were administered through water compared with the levels seen using other administration protocols, perhaps because of continuous drinking in the aquatic environment [39–42].

In Table 1, the information related to different probiotics administered only through water is summarized.

Table 1. Summary of probiotics influences on different finfish species when they were administered solely through water.

Probiotics	Biological Model	Concentration	Fish Density, Age, Average Weight	Inoculation Times	Contact Duration	Major Outcomes	References
Lactobacillus plantarum (later classified as *Carnobacterium divergens*)	Atlantic cod (*Gadus morhua* L.)	10^5 CFU mL^{-1}	5 dph	-	9 days	Strain colonization Decreased opportunistic bacteria	[24]
Vibrio pelagius	Turbot (*Scophthalmus maximus* L.)	4×10^5 bacteria mL^{-1}	0 dph	-	14 days	Influenced gut microbiota	[31]
V. pelagius and *Aeromonas caviae*	Turbot	10^5 bacteria mL^{-1}	2–8 dph	-	16 days	Colonization of probiotic bacteria in the group received the bacteria at earlier days. Increased survival in group received *V. pelagius*	[32]
C. divergens and *V. pelagius*	Turbot	10^5 bacteria mL^{-1}	0 dph		15 days	Higher colonization of *V. pelagius*. No significant difference in survival	[33]
Enterococcus faecium ZJ4 (isolated from intestinal tract of piglet)	Tilapia (*Oreochromis niloticus*)	10^7 CFU mL^{-1}	30 fishes (with average weight 6.83 ± 0.18 g) per aquarium	Every 4 days	40 days	Significantly higher final weight, DWG, complement component 3 content, MPO activity and RBA of blood phagocytes in group treated with probiotic ($p < 0.05$). No significant difference in total serum protein, albumin and globulin concentration and lysozyme activity between treated group and control	[43]
Pseudomonas sp. (isolate GP21) isolated from the intestine of Atlantic cod	Atlantic cod	3.8×10^8 CFU mL^{-1}	8 fishes (with average weight 150 g) per tank	-	1 h	Downregulated *defb* expression in gills and upregulated *defb* expression in skin after treatment with probiotic	[44]
Commercial probiotic Fishery Prime™ (Keeton Industries, Wellington, CO, USA)	*Perca flavescens*	5 g to each 100 L tank	30 fishes (with average weight 6.17 ± 2.27 g) per tank	Every day	6 weeks	Significantly higher weight gain ($p < 0.05$) and higher levels of GH and IGF-I transcription in group treated with probiotic	[45]
Paenibacillus polymyxa	*Cyprinus carpio*	10^3, 10^4 and 10^5 CFU mL^{-1}	20 fishes (with average weight 23.17 g) per tank	3 times a week	8 weeks	Significantly improved survival, lysozyme activity, MPO content, RBA, catalase and superoxidase dismutase activity and resistance against *Aeromonas hydrophila* in groups treated with probiotic ($p < 0.05$)	[46]
Bacillus licheniformis Dahb1	Asian catfish (*Pangasius hypophthalmus*)	10^5 and 10^7 CFU mL^{-1}	12 fishes (with average weight 15 ± 2.5 g) per group	-	24 days	Significantly higher weight gain, survival (with no mortality during contact time), RBA, GST activity, total glutathione activity ($p < 0.05$) and higher MPO and lysozyme activity in group treated with probiotic. No significant difference in ACH_{50} and GR in group treated with probiotic	[25]
Vibrio lentus	Sea bass (*Dicentrarchus labrax*)	10^6 CFU mL^{-1}	12 larvae (4, 6 and 8 dph) inoculated via immersion in well plate	-	-	Significantly modified gene expression (i.e., immune response, cell proliferation and death, cell adhesion, ROS metabolism and iron transport ($p < 0.05$). No significant differences in apoptotic and cell proliferative indexes	[47]

CFU: colony forming units, DWG: daily weight gain, MPO: myloperoxidase, h: hour, *defb*: *beta defensin*, RBA: respiratory burst activity, GH: growth hormone, IGF: insulin-like growth factor, dph: days post hatching, ROS: reactive oxygen species, ACH_{50}: serum natural complement hemolytic activity, GST: Glutathione-*S*-transferase, GR: reduced glutathione.

The results obtained from this method are similar to those achieved by other administration routes. According to the published literature, probiotics as water additive could have several favorable effects on finfish aquaculture, although two main advantages have been especially emphasized: (i) the ability to control the quality of water by bioremediation, and (ii) the biocontrol of pathogens through antagonistic effects [48].

3.1. Improvement of Water Quality

Ammonium and nitrite, toxic metabolites originating in the feces, underused feed, and waste in aquatic systems can result in enormous economic loss [49,50], since they can affect the physiology, immunity, survival, and growth of animals [51,52]. Traditionally, toxic metabolites have been controlled by biofilters and water exchange [53–55]. Specifically, in re-circulating aquaculture systems (RAS), parameters of water quality need to be regularly controlled [56]. The zero water exchange (under sufficient management of carbon:nitrogen ratio) leads to an accumulation of organic matter and nutrients in aquaculture systems. It normally develops the microbial community, and the high diversity of microorganisms promotes the stabilization of the system by taking the nitrogen compounds which generate in situ microbial protein [57,58], improvement of nutrition [59–61], reduction of food conversion ratio (FCR), and feed costs, besides promoting the health of the organisms which are cultured [62,63]. In nature, the control of toxic compounds (ammonium and nitrogenous compounds) is potentially carried out by denitrifying bacteria; this is a role which probiotics might play. Recently, different kinds of probiotics have proven effective in ammonia nitrogen degradation. Accordingly, these eco-friendly additives can contribute to improve water quality [64]. For example, *Bacillus subtilis* has been widely administered as an appropriate probiotic agent to control the water quality [65]. The administration of *B. subtilis* as a water additive in the rearing water of olive flounder (*Paralichthys olivaceus*) resulted in significantly reduced ammonia levels and fish mortality [66]. Gram-positive bacteria, especially *Bacillus* spp., have been able to convert organic substances to carbon dioxide more efficiently when compared with Gram-negative bacteria converting a larger amounts of organics into bacterial biomass or slime [12,67,68].

Probiotic use in the rearing water to improve water quality, and their administration to purify waste water from fish farms is helpful in areas with decreasing surface water, since the water can be reused for aquacultural activities after treatment. The probiotic bacterium *Bacillus licheniformis* isolated from large yellow croaker (*Pseudosciaena crocea*) not only significantly decreased ammonia levels, but also the starch and protein from underutilized feed in waste water [69]. A sustainable community development (SCD) study on probiotics technology for purifying waste water in ponds indicated an 85.2% decrease in ammonium nitrate concentrations, as well as a decreasing tendency in chlorophyll a levels [70]. In fact, probiotics can increase the composition of microbial species in water and improve its quality [12]. Furthermore, the higher quality of water after the addition of probiotics into aquatic environment might be because of the control of the parameters, such as dissolved oxygen, pH, and temperature. [71,72]. However, using some probiotic species (i.e., *Bacillus*, *Rhodopseudomonas*, *Nitrobacter*, *Cellulomonas*, *Pseudomonas*, and *Enterobacter*) in the cultivation environment of channel catfish was not successful in improving water quality; consequently, except for nitrification, publications investigating the effects of probiotics on water quality are limited [1,73], and understanding of the mechanisms of action is still in its infancy [74].

3.2. Inhibitory Activity against Fish Pathogens

Probiotics are considered effective at strengthening innate immune responses, owing to the fact that they can interact with natural killer cells, monocytes, macrophages, and neutrophils. Their ability to enhance the number of macrophages, lymphocytes, erythrocytes, and granulocytes of a variety of fishes has been reported [27,75–79]. Dead probiotics are believed to have several advantages, as well. Such safe products can be applied to modify the biological responses [80]. Thus, probiotics can defend the host against various infections caused by pathogenic microorganisms [81]. The control of streptococcosis, one of the main problems in tilapia culture, has been observed as a result of enrichment of artificial feed by probiotic [82]. Although reinforcement of fish immunity and strength has been demonstrated in several studies, applied oral administration of probiotics, using probiotics as water additives is the appropriate recommended method in some specific areas:

3.2.1. Improvement of Non-Specific Defense in Marine Fish Larvae

Because of continuous exposures of fish skin to the aquatic environment, mucosal epithelia are considered a first-line defensive organ. A repertoire of immune cells and molecules (innate and adaptive) characterize the immune system related to mucosal epithelia of fish, governed by skin-associated lymphoid tissues, gill-associated lymphoid tissues, and gut-associated lymphoid tissues [83–88]. Marine fish larviculture has proven challenging, with high mortalities due to many complex and unknown causes; however, since the specific immune system is not completely mature during the early weeks after hatching, microorganisms may be an important cause of epizootic mortality [89–91]. Probiotic administration through incubation water can improve and stimulate the non-specific defense, as the epidermal mucus layer comprises the primary interface between larvae and the aquatic environment [29]. A significantly higher larval survival rate following the addition of the probiotic bacterium *L. plantarum* to the incubation water of Atlantic halibut (*Hippoglossus hippoglossus* L.) eggs and larvae was confirmed [91].

3.2.2. Treatment of Fungal Infections

Saprolegniosis, one of the major diseases in freshwater environments, is caused by fungal infections, and it can cause serious economic losses in fish farms [92,93]. Several antifungal drugs have been used to treat this disease, as mentioned in different studies. The inhibitory effect of the probiotic strain *Aeromonas media* A199 on *Saprolegnia parasitica*, which causes serious winter infection, has been shown in two fish species: eel (*Anguilla australis*) and silver perch (*Bidyanus bidyanus*), under experimental conditions [94–96]. In all of these studies, the addition of a probiotic to the water column resulted in hyphal matter release from skin into the water.

3.2.3. Improving the Substrate Spawners Aquaculture

To ensure a sustainable aquaculture and the safe breeding of substrate spawner species, the protection of egg clutches against pathogens is a vital necessity. Currently, no commercial therapeutics specialize in this field, especially in the case of marine species. The use of probiotics is highly promising in attempts to inhibit pathogenic infestations and decrease the mortality of larvae. Functionalized spawning tiles with probiotic *Pseudoalteromonas* biofilms designed for clown fish have proven to be promising candidates for the prevention of egg clutches from pathogenic infestation. Thus, probiotic application in water media can pave the way for improving the state of substrate spawners' aquaculture. Indeed, pathogenic wash out from the tiles was achieved as a result of the antagonistic effects of probiotics [97].

4. The Major Factors Regulating the Benefits of this Administration Method

Treatment duration (contact time), dose, and source of probiotics are important factors that can affect their activity [2,98,99]. However, the main factors that can affect the benefits of probiotics administration through water are outlined below.

4.1. Temperature

Temperature appears to be an influential factor on therapeutic activity of probiotics in aquaculture systems when they are administered through water. To exemplify this, the maximum antagonistic activity of the probiotic strain *A. media* A199 against saprolegniosis disease was attained with a minor water temperature increase [96].

4.2. Treatment Dose

The beneficial dose of probiotics used by direct addition to the water medium must be calculated according to the weight of the treated fishes, as well as the water volume. In a study, the effects of the commercial probiotic SA-NOLIFE MIC-F®, INVE Aquaculture (Dendermonde, Belgium) (a mixture of *B. licheniformis*, *B. subtilis* and *Bacillus pumilus*) on growth performance and immune defense in *Perca fluviatilis* L. larvae were investigated. However, the administration of bacteria through water did not give rise to any significant increase in growth, survival, and immunity. It is possible that probiotic uptake by fishes with this administration mode was not sufficient to stimulate their digestive mechanisms [100]. Likewise, the direct addition of *Lactobacillus* spp. as a probiotic to the tank water of gilthead sea bream (*Sparus aurata* L.) larvae did not result in any significant increase in growth and digestive enzyme activities compared to probiotic administration in live food [101].

4.3. Inoculation Times

To have a stable population of probiotics at a final calculated concentration, inoculation must be renewed during treatment time. The times that the probiotic should be inoculated into the water will depend on the length of time a given probiotic takes to disappear from the water column. For this reason, this time should be calculated before the beginning of treatment.

4.4. Age of Treated Fishes

It has been demonstrated that probiotic administration at the earlier larval stages can modulate the gut microbiota more effectively, as the digestive tract is not completely developed, and the established microbiota reflect that of the feed and rearing water. Hence, early probiotic treatments through rearing water are strongly suggested for maximum effect. Administration of two probiotic strains (*Arthrobacter* sp. and *Enterococcus* sp.) via water column (10^5–10^7 CFU mL^{-1}) at the early post-fertilization of cod could control the endogenous microbiota, and contribute to the enhancement of larval growth and survival [102].

4.5. Salinity

Salinity is claimed to decrease the survival of probiotic bacteria, and it may limit their application in sea-water. The half-lives of LAB in sea water (35 g L^{-1}) was reported to be between 3–21 h (at 20–23 °C) [103]. This short duration of viability might culminate in lower colonization of probiotics in fish species inhabiting environments with higher salinities. However, in vitro and in vivo study of eight LABs, isolated from fish and seafood, as potential probiotics for turbot farming revealed that all isolates could survive in sea water for seven days (at 18 °C) [10]. It looks as if salinity and temperature affect the beneficial effects of probiotics concomitantly. On the other hand, it must be mentioned that, in general, the direct addition of probiotics to the water column has been considered more effective than other methods in marine aquaculture [39–42].

Besides the factors outlined above, it seems as if the effects of probiotics are highly varied among various fish species [81], although there is not sufficient evidence to substantiate that.

5. Research Gaps and Future Perspectives

Huge benefits in aquaculture have been obtained by using probiotics as alternatives to antibiotics and chemicals, so this achievement merits more research in order to study related but less investigated issues. One of these issues is the effectiveness of different administration methods. This issue can be considered a key factor in the modern generation of probiotics in the aquaculture industry. The direct addition of probiotics into water column in aquaculture systems as a potential method has been less investigated compared to the other application methods, although some studies point to the high efficiency of probiotic administration through water. Furthermore, as this method is more effective in marine environments because of higher probiotic uptake by treated fish (on account of intensive drinking activity in these environments), more research regarding probiotics administration through water in different marine fish species should be carried out. Further studies in terms of environmental safety of these additives seem to be needed [104].

The least investigated issues in the field of probiotics administration as a water additive include the relationship between the quality and quantity of probiotics needed to control ammonia nitrogen in aquatic environments [49,105]. Likewise, despite the beneficial effects of yeasts as probiotic in finfish aquaculture, no information is available on their administration in the rearing water.

The application of different probiotics in biofloc systems has proven to be effective in the improvement of shrimp production [106,107]. To the best of our knowledge, no studies have been conducted to investigate the effects of biofloc technology combined with probiotics in finfish aquaculture.

Research into this administration route should include different molecular biotechnology tools. DNA microarrays are widely used for the evaluation of immune responses as well as dietary effects in fish [46,108,109], but the information addressing transcriptomic effects stimulated by probiotics administration through water is very limited; accordingly, further studies should focus on this novel tool to gain a better understanding of the efficacy of probiotics application as a water additive.

6. Conclusions

Administration of therapeutics and feed additives in aquatic environments has its limitations in comparison to terrestrial habitations; that is the reason why the strategies which are applied in this area have been the topic of several studies related to aquaculture. Among these topics, finding the most effective and affordable approach has been a key focus. Probiotics as an alternative to chemicals and antibiotics have proven to be effective in promoting successful aquaculture, as they have the potential to improve water quality, increase tolerance to stress, generate high-quality livestock etc. Regarding all of these benefits, the routes of probiotic administration need to be more investigated. Our brief review suggests that the best administration method should be selected according to age and size of fish, aquaculture system, and all other contributing factors. Although the direct addition of probiotics to the water has been shown to be effective in different studies, it cannot be proposed as the best way in all cases where probiotics are used.

In conclusion, further research in the field of probiotic administration through water needs to be conducted in order to develop economically acceptable treatment practices for intensive production, always taking into account that the results may vary according to the different probiotics used.

Author Contributions: Funding acquisition, M.Á.E.; Review, L.J. and M.Á.E.; Writing—original draft, L.J.; Writing—review & editing, L.J. and M.Á.E.

Funding: This research was partly funded by the Spanish Ministry of Economy and Competitiveness (MINECO) co-funded by the European Regional Development Funds (Grant no. AGL2014-51839-C5-1-R) and the *Fundación Séneca de la Región de Murcia* (Grupo de Excelencia grant no. 19883/GERM/15).

Conflicts of Interest: the authors declare no conflict of interest.

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