Host-derived Probiotics for Finfish Aquaculture

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Abstract. The mucosal surface of aquatic animals harbors a diverse number of microorganisms with interesting biological and biochemical characteristics. Besides acting as a natural defense system of the host, the microbial community that is associated with these specialized tissues and organs serves as reservoirs of candidate probiotics. The use of probiotics is a strategy employed to improve the health of the host and to prevent infectious diseases. Terrestrial microorganisms that act as natural defense systems of most cultured species have been identified as the main producer for these beneficial bacterial candidates. At present, there are a number of commercially available probiotics but their benefits in large-scale aquaculture operations are largely variable. Host-derived probiotics have gained popularity in recent years as they are alternative sources of beneficial microbes to the aquaculture industry that is primarily dependent on the use of terrestrial microorganisms. The relevance of host microbiota and its potential as a source of candidate probiotics are demonstrated in a number of studies done in various fish models. By presenting the results obtained from previous studies on the ability of host-associated probiotics to improve growth and health of the host, this review condensed the current knowledge and information in order to provide a platform for future research and development on the application of host-associated probiotics in finfish aquaculture.

Keywords: beneficial microorganisms, mucosal immune response, probionts, prophylaxis

1. Introduction

Aquaculture is an important economic activity in most developing countries and has expanded and intensified in recent years. This industry has an important role in providing livelihood opportunities and food for the growing population [1]. Due to intensification in aquaculture practices, the emergence of a wide array of pathogens resulted in infectious diseases to be the major limiting
factor in most aquaculture operations [2]. To control the infectious diseases in aquatic organisms, a number of chemicals and antibiotics have been regularly used in most aquaculture facilities. These antibiotics are not only used for the treatment of bacterial infections, but are also applied for preventing these infections [3]. In addition, the use of antibiotics and chemicals is a partially effective strategy for disease management [4]. While their application in hatcheries may be cost-effective due to the limited water volume, its use in grow-out systems with large-volume water exchange is costly; thus, avoided by most fish farmers [5]. However, it is widely known that the application of these compounds may be detrimental to the environment and poses serious risk to human health. It can result in the development and transfer of resistance to other bacteria, including human and fish pathogens [6]. Thus, the development of natural and environment-friendly alternatives has become important and urgent for the production of healthier aquatic animals. Examples of these alternatives include the use of vaccines, immunostimulants and probiotics [7]. This mini-review provides a brief overview on the development and use of host-derived probionts in aquaculture. In particular, this will focus on the fish species where these probionts have been isolated and their modes of action in the host.

2. Probiotics: history and definition in the aquaculture context

The term probiotics came from the Greek words “Pro” and “bios” meaning “for life” [8]. It helps to improve the overall health of the host organism in a natural way and within the context of disease control, the use of probiotics anchors on the principle of “microorganisms against microorganisms” [9]. Probiotics has been initially defined as organisms or substances that contribute to intestinal microbial balance [10]. It was then defined by Fuller as a live microbial feed supplement having ability to improve the microbial balance of host animal in 1989 [11]. A joint expert panel from Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), defined probiotics as “live microorganisms, which when consumed in adequate amounts, confer a health benefit for the host” [12].

From a historical standpoint and available literatures, the origin of using probiotics in aquaculture is not clear but there is sufficient evidence that demonstrated on the use of probiotics in extensive finfish and invertebrate culture in China and India [13]. The term “probiotics” was first used [14] to describe ‘substances secreted by one micro-organism which stimulates the growth of another’. Their definition of probiotics provided the basis of differentiating probiotics from antibiotics. An earlier study [15] in the mid-1980s involving the use of Bacillus toyoi spores as feed additive to increase the growth rate of yellow tail, Seriola quinqueradiata demonstrated the potential benefits of using probiotics in aquaculture. Unfortunately, that study did not stimulate further research initiatives along that line because very few published studies were documented thereafter [9]. It was in the late 1990s that probiotics research became prominent in aquaculture and by the early 2000s, Verschuere and colleagues proposed an aquaculture-based definition of probiotics, which states “A probiotic is defined as a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment” [16]. This definition allowed a broader application of the term “probiotic” by encompassing the role of probiotics in aquaculture and the aquatic environment. The recent definition also addressed the limitations posed by Fuller’s definition of probiotics, particularly in clarifying the complex interactions among probiotics, the culture environment and the aquatic animal.

The complex nature of the aquatic environment makes it difficult in formulating a unified definition of probiotics; thus, their definition is based largely on their modes of action on a case-by-case basis [17]. It even becomes more complicated if the following concepts are introduced, such as: a) direct application of the probiotics to the rearing water [18] and b) bacterial viability [19, 20, 21]. The unified definition of a probiotics must encompass the physico-chemical and biological prerequisites of a dynamic aquatic system including the organisms that live in it. In response to the points raised [22], a simplified yet broad definition of probiotics was proposed [17] stating that
probiotics are “live or dead, or even a component of the microorganisms that act under different modes of action in conferring beneficial effects to the host or its environment”. According [23], probiotics can improve disease resistance, growth performance, feed conversion ratio (FCR) and stress resistance in aquatic organisms. It is important to point out that the effects of probiotics in finfish aquaculture are not limited to the intestinal tract of the host, but can also improve the health of the host by controlling the proliferation of pathogens and improving water quality through modification of the microbial community in the rearing water and sediment [16].

Probiotics are beneficial for the host because they are able to persist in the digestive tract due to their tolerance to acid and bile salts [24]. The use of probiotics has gained increasing scientific and commercial interest and is now quite commonly used in developing health - promoting foods to therapeutic, prophylactic and growth supplements for fish [8]. Proof of their increasing popularity is evidenced by the isolation and evaluation of probiotic candidates for use in aquaculture in the prevention and control of infectious diseases. Some of these have been isolated from aquatic animals including finfish and shrimps, their digestive tract, culture environment, terrestrial animals and other sources [13]. In the global context, no international standard and regulatory guidelines are strictly enforced regarding the use of probiotics in aquaculture [25]. As a result, there are quite a number of commercially available probiotics that are being used in finfish aquaculture (Table 1). The various genera that comprise these probiotics, which are sold in the market include Bacillus, Carnobacterium, Lactobacillus, Enterococcus, Pseudomonas, Shewanella, Streptococcus and Vibrio. They are used in the culture of both cold- and warm-water fish species at their various life stages.

Table 1. Commercial probiotics for finfish aquaculture. Modified from Shefat (2018).

| Bacterial species                  | Fish species                                    |
|-----------------------------------|------------------------------------------------|
| 1. *Bacillus* sp.                 | Catfish                                        |
| 2. *Bacillus subtilis*            | *P. olivaceus*                                 |
| 3. *Carnobacterium divergens*    | *G. morhua*                                    |
| 4. *Enterococcus faecium*        | *A. anguilla*                                   |
| 5. *Lactobacillus acidophilus*   | *C. garstina*                                  |
| 6. *Lactobacillus rhamnosus, Pseudomonas fluorescens, Vibrio fluvialis, Pseudomonas* sp. | *Oncorhynchus mykiss* |
| 7. *Lactococcus lactis*          | *Epinephelus coioides*                         |
| 8. *Lactobacillus delbrueckii*   | *Dicentrarchus labrax*                         |
| 9. *Lactobacillus helveticus, Streptococcus, Scopohiobius maximus, thermophilus* | *Solea senegalensis*                           |
| 10. *Shewanella putrefaciens*    | *Saimdalis*                                    |
| 11. *Vibrio alginolyticus*       | *Salmo salar*                                   |

3. Selection and use of host-derived probiotics in aquaculture

Probiotics was primarily considered a disease control agent in aquaculture [9] and this context, earlier studies on the modes of action of probiotics in fish clearly demonstrated the direct inhibition in the growth and proliferation of pathogens and in the stimulation of beneficial microbiota of the host and its rearing environment [26,27]. Therefore, the selection of appropriate probiotics particularly by taking into consideration the physiological and biochemical features of the host and the physico-chemical conditions of the immediate environment is crucial [9]. Interestingly, several other beneficial properties have been documented in probiotics hence expanding the understanding of their use in farmed aquatic animals. The application of probiotics has a significant advantage not only by being a
disease control agent but notably as alternatives that promote health and welfare in farmed animals in a larger scope [17]. Aside from their role in modulating immune responses in the host, probiotics are thought to have diverse modes of action in living organisms. For example in terrestrial animals, they competitively exclude pathogens by producing inhibitory molecules or through direct competition for space, nutrients or oxygen in the digestive tract of the host [22]. In aquatic animals, probiotics including *Brevibacillus brevis*, lactic acid bacteria, *Vagococcus fluvialis* and *Vibrio harveyi* attach to the mucosal epithelium of gastrointestinal tract of fish in order to resist pathogens [28,29]. However, in another study it was revealed that probiotics do not attach and colonize the digestive tract of fish [30]. Whether these conflicting reports are due to the research methodologies that were used need to be carefully studied and analyzed. Another mode of action of probiotics is their ability to enhance the digestion [31] through the production of enzymes such as amylases and proteases [32,33]. They also improve the health status of the host by stimulating the production of nutrients including fatty acids, biotin and vitamin B12 [34,35, 36].

The mechanisms of action of probiotics in aquaculture are not yet fully elucidated [13]. However, their presumed modes of action are based on the number of studies done in various species of fish [37,38,39,40,33]. First, they facilitate competition for binding sites. It is also known as “competitive exclusion”, where probiotic bacteria attach to the binding sites in the intestinal mucosa resulting in the formation of a physical barrier, which prevents entry of the pathogenic bacteria. Second, they enable production of antibacterial substances. Probiotic bacteria synthesize compounds like hydrogen peroxide and bacteriocins, which have antibacterial action on pathogenic bacteria. They also produce organic acids that lower the pH in the gastrointestinal tract. This prevents the growth of various pathogens but triggers the development of certain species of *Lactobacillus*, which are beneficial to the host. Third, they compete with the pathogens for utilization of nutrients. Probiotics compete with the harmful pathogens for nutrition absorption; thus, reducing the amount of nutrients for the latter. The lack of nutrients for the pathogenic bacteria is a limiting factor for their maintenance. Lastly, they stimulate the various components of the immune system. Some probiotic bacteria are directly linked to the stimulation of the immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon [9,23].

Endogenous microbiota is an important component of the mucosal barrier, which acts as the first line of defense against various pathogens [41]. Recently, the use of host-associated microorganisms as probiotics in aquaculture has gained much attention [9], though it remains unclear whether host-associated bacteria are better than those obtained from exogenous sources [42]. However, it is believed that host-associated microorganisms may be more suitable and would have optimal benefits in similar environments. There is a wide range in the variation of the taxa present in the endogenous microbiota of both marine and freshwater species, which can be influenced by genetic, nutritional and environmental factors, however, members of the genera *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Carnobacterium*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Photobacterium*, *Pseudomonas* and *Vibrio* constitute the dominant microbiota of these marine species [43,44]. It has been reported in freshwater species the presence of *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Lactococcus* and *Pseudomonas*, representatives of the family *Enterobacteriaceae*, and obligate anaerobic bacteria of the genera *Bacteroides*, *Clostridium* and *Fusobacterium* [45,46]. Probiotics are usually members of the healthy microbiota associated with the host; therefore, they may provide an alternative way to reduce the use of antibiotics in aquaculture [10]. Probiotics may prevent bacterial diseases through a variety of mechanisms, such as the creation of a hostile environment for pathogens by producing inhibitory compounds, by competing for essential nutrients and adhesion sites or by modulating the immune responses [22].

The selection and evaluation of probiotic candidates for aquatic species are anchored on these five principal ideas, namely, isolation, microevaluation, application, macroevaluation and commercialization [16, 9]. Isolation, refers to how and where the candidate microorganism/s is/are isolated. Microevaluation refers to the identification and in vitro characterization of the probiotic properties of the candidate microorganism/s. Application refers to the appropriate mode of delivery of
the candidate microorganisms to the host. Macroevaluation, refers to the identification of the beneficial effects to the host and to its immediate environment during administration of the probiotic candidate/s. Commercialization, refers to the large scale production of probiotics and determining economic feasibility and viability. Proper isolation provides the solid foundation for the ultimate output which is "commercialization" [9]. Therefore, it is necessary that the source of the probiotic candidate/s must be carefully assessed as this has a significant bearing on the eventual success of their application.

In recent years, there is an increasing interest towards using the host microbiota as a source of probiotics. The host microbiota acts as a natural defense system of the host [47] and it possesses a number of beneficial factors [48,33]. Fish species that are being used in aquaculture are a rich reservoir of probiotic strains, which can be isolated and tapped to protection against some infectious diseases [49]. Host-associated microorganisms have special roles aside from being commensals of aquatic animals. They are able to colonize the gut of animals and are able to perform crucial functions for the host [50]. They have significant roles in development and provide protection to the gut, which earned them the distinction of being an "extra organ" of the host [51].

The host-associated microorganisms that are found in mucosal surfaces of finfish are a good source of probiotics for the aquaculture industry that has been too inclined to using terrestrial probiotics. These mucosal surfaces harbor a rich diversity of microorganisms with a variety of functions, and potential probiotics candidates are isolated from these mucosal tissues [11, 40, 24]. The gut of fish is not just an important mucosal organ but also provides a niche for adherence, colonization and proliferation of many microbial species that affect various physiological processes of the host [52, 41]. The fish intestinal microbiota is highly influenced by the host ecology and its environment [53]. The gut, being one of the few organs with defined and multiple functions [54] can be a good source of potential probiotics [9].

From a number of previous studies, Table 2 shows the different host-derived probiotic candidates that have been isolated from fish and their properties including their effects on various pathogens. Most of the host-derived probiotic candidates were obtained from the gastrointestinal tract of marine fish species. These probiotic candidates belong to the genera Lactobacillus/Bacillus, Enterococcus, Pseudomonas, Psychrobacter, Shewanella, Vibrio, Alcaligenes and Acinetobacter. They also showed antagonistic activities against some bacterial pathogens of fish.
Table 2. Isolation and modes of action of selected host-associated probiotic candidates from finfish.

| Bacterial species | Fish species and source of isolation | Effects on host | Reference |
|-------------------|--------------------------------------|-----------------|-----------|
| *Psychrobacter* sp., *Shewanella* sp., *Photobacterium* sp. and *Vibrio* sp. | Atlantic cod (Gadus morhua)/gastrointestinal tract | inhibition of *Vibrio anguillarum* and *Aeromonas salmonicida* | Cai et al. 2010 |
| *Pseudomonas* sp. and *Psychrobacter* sp. | Atlantic cod (Gadus morhua)/gastrointestinal tract | modulating the transcriptional immune responses in; enhanced production of digestive enzymes in the intestinal epithelial cells; inhibition of *Vibrio anguillarum* and *Aeromonas salmonicida* in intestinal epithelial cells | Loza et al. 2010a, b, 2011, 2012 |
| *Lactococcus* spp. | Rainbow trout (Oncorhynchus mykiss)/microbiota and rearing water | inhibition of *Lactococcus garrigueae* | Araujo et al. 2015 |
| *Enterococcus* casseliflavus | Rainbow trout (Oncorhynchus mykiss)/intestines | improved immunity; enhanced digestive enzymes; resistance against *Streptococcus iniae* infection | Safari et al. 2016 |
| *Lactococcus* lactis | Japanese flounder (Paralichthys olivaceus)/gastrointestinal tract | protection against *Streptococcus parasitica* through competitive exclusion; increased innate immune responses; improved growth; higher serum and gut metabolites | Nguyen et al. 2017, 2018 |
| *Alcaligenes* sp. | Malaysian Mahseer (Tor putitora)/gut | enhanced nutrient utilization and metabolism through increased gut surface area; increased short-chain fatty acid production; modulation of gut microbiota | Asaduzzaman et al. 2018 |
| *Lactobacillus* plantarum and *Bacillus* velezensis | Nile tilapia (Oreochromis niloticus)/intestines | improved mucosal and serum immunity; resistance against *Streptococcusagalactiae*, enhanced growth performance | Van Doan et al. 2018 |
| *Psychrobacter* sp., *Staphylococcus saprophyticus* , *Acinetobacter baemalgalactus* | turboid/gastrointestinal tract (Scophthalmus maximus) | no effect on digestive enzymes, growth, resistance against bacterial infection | Wenke et al. 2019 |
| *Bacillus* pumilus | golden pompano (Trachinotus ovatus)/gastrointestinal tract | improved growth; enhanced expression of immune-response genes in the gut; higher survival following infection with *Vibrio anguillarum* | Liu et al. 2020 |
4. Conclusion and future perspectives

The use of probiotics in aquaculture is still faced with a lot of controversies and skepticism due to lack of authentic evidence or real environment demonstrations on the successful use of these probiotics and their mechanisms of action. The modes of actions have been largely speculative and based on laboratory studies. The effects of probiotics in commercial aquaculture have not clearly elucidated on the exact modes of action that resulted in increased production or improved health status of the fish. The probiotics may either exert a direct or indirect effect, but which one of the factors that had significant contribution cannot be accurately determined. Nevertheless, a review of available literature demonstrated the promising effects of probiotics particularly on their roles in providing disease resistance in fish against various infectious agents. Hence, it is likely seen that oral administration of probiotics via incorporation in the diets will receive increasing attention as an alternative for antibiotic in aquaculture.

Because the exact nature of the modes of action of probiotics in fish is not totally understood, further research on probiotics should focus on the different molecular biotechnology tools that will aid in a better understanding of the modes of action of these microorganisms in the host. Various molecular tools such as immunohistochemistry, gene expression and proteomics can be used to explore the mechanisms of actions of these host-derived probionts. Research studies should also focus on how these host-derived probionts modulate the gut microbiota and how these probionts survive as they pass through the different regions of the gastrointestinal tract in order to adhere and colonize these sections must be elucidated in future studies as well.

Based on available scientific studies, it is difficult to draw a solid conclusion on whether host-associated microorganisms are better than those of non-host origin. One research thrust that might be of great interest in the future is to try the approach of creating a probiotics cocktail that combines both the host and non-host candidate microorganisms during probiotic application in an aquaculture facility. This can be done on the condition that these microorganisms are not inhibitory or antagonistic with one another; hence, favorable benefits are likely to be manifested by the host during probiotics application. In fact, earlier studies have pointed out that the functionality and efficacy of the probiotics are greatly enhanced using multi-strain organisms [55,56]. These studies also stressed that the host-associated microorganisms have other uses aside from being involved in the natural defense system. [48] have shown that the diverse microbial community of the fish gut is a good source in discovering novel natural products. It should be borne in mind that the effects of probiotics in finfish could be species-specific. Therefore, the mechanisms by which host-associated probiotics act in vivo warrant further research. It is also important that the interactions between the probiotic bacteria and the gastrointestinal microbiota of the host be investigated. These kinds of studies will enable us to establish efficient selection criteria of further developing these candidate microorganisms that are not harmful to the host but ensure good growth and a robust immune system.

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