PROBIOTICS IN AQUACULTURE AND THEIR APPLICATIONS AGAINST PATHOGENIC BACTERIA

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Abstract

Probiotics play important role in improving the public health by enhancing the internal microbial balance. Probiotic bacteria produce siderophores, bacteriocins, lysozymes, hydrogen peroxides and proteases, which can inhibit pathogenic bacteria. Also, some beneficial bacteria provide amino acids, vitamins, enzymes and fatty acids. *Bacillus subtilis*, *Aeromonas* spp., *Bacteroidaceae*, *Lactobacillus plantarum*, *Clostridium* spp., and *Staphylococcus* sp. produce amylase, but *B. subtilis* and *Staphylococcus* sp., *L. plantarum* produce protease, while *L. plantarum*, *B. subtilis*, and *Staphylococcus* sp. produce cellulase. In aquaculture, probiotics have different health benefits and play key roles in increasing growth rate, improving disease resistance, immunity, gut microbiota, and in enhancement of epithelial barrier integrity. Furthermore, the uses of probiotics additives in fish diets could minimize the side effects of antibiotic growth promoters. Promoting the application of probiotics for fish would help improve the production, reproduction and feed efficiency and, therefore, safeguard consumer health as well. This article reviewed the definitions, probiotic use in fish rations against pathogens and potential uses of probiotics in aquaculture.

Key words: Fish, Health, Nutrition, Pathogenic bacteria, Probiotics

Introduction

Aquaculture has been found to be a key cause of antibiotic contamination in areas where antibiotics are used (Rico *et al.*, 2014). One possible solution to the problems of bioaccumulation and antibiotic resistance that aquaculture faces, is the use of nutraceuticals and feed additives (Abd El-Hack *et al.*, 2016; Alagawany *et al.*, 2020 a,b,c; 2021). The term nutraceutical is a compound word that combines nutrition and pharmaceutical. Therefore, nutraceuticals can be defined as “substances that have physiological benefits or provide protection against chronic diseases” (Hamid *et al.*, 2014). There are different types of nutraceuticals, like

phytochemicals and dietary supplements, and one important category is tailored food (Hamid *et al.*, 2014; Abdel-Latif *et al.*, 2020; Naiel *et al.*, 2020a,b; Mahmoud *et al.*, 2021). Tailored foods can be defined as “any food or ingredient that has a positive effect on an individual’s health, performance, or state of mind” (Hardy, 2000). There are different types of functional foods. Prebiotics and probiotics are two emerging types of feed additives that have shown promise for use in fish production (Schmidt *et al.*, 2017; Wang *et al.*, 2019; Elgeddawy *et al.*, 2020; López Nadal *et al.*, 2020; Ringø *et al.*, 2020; Sharif *et al.*, 2021).

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Probiotics were originally defined as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989). A more modern definition is “Live microorganisms which, when administered in adequate amounts confer a health benefit on the host,” (FAO & WHO, 2006; Alagawany *et al.*, 2016, 2018; Soomro *et al.*, 2019). Probiotics work by supporting and complementing the host’s natural gut microbiota and they can accomplish this through a variety of means, and safeguard health of fish and aquaculture species from various aspects including of potential antimicrobial and biocontrol agents (Bentzon-Tilia *et al.*, 2016; Zorriehzahra *et al.*, 2016; Banerjee and Ray, 2017; Hoseinifar *et al.*, 2018; Noor *et al.*, 2020; Ringø *et al.*, 2020). The present review describes definitions of probiotics and use of probiotic in aquaculture as well as its use against pathogenic bacteria in fish aquaculture.

Definitions and use of probiotics in fish production

In literature, there are many variations in the definition of probiotics and most of them are specific to a health benefit conferred on the gut of the host (Gatesoupe, 1999). Historically, many of these definitions are associated with the gram-positive lactic acid bacteria (LAB) administered to humans and terrestrial animals. The increasing interest in farmed fish and shellfish has also led to investigations of potential probiotic use in the aquaculture industry. The use of probiotics in intensive larviculture of various marine fishes could be a promising future strategy, as indicated by evidence-based approaches (Tarnecki *et al.*, 2019; Wang *et al.*, 2019; Kuebutornye *et al.*, 2020; Noor *et al.*, 2020; Ringø *et al.*, 2020).

Bacilli have been observed to improve the survival of reared shrimp and control the luminous *Vibrio* spp. (Moriarty, 1998). *Bacillus* sp. can also protect the live-feed (*Artemia*) and increase survival by reducing the activity of *Vibrio*

campbellii and enhancing the innate immune response of the artemia larvae (Niu *et al.*, 2014). Defoirdt *et al.* (2011) isolated *Bacillus* spp. from white leg shrimp and European sea bass, which could degrade N-acylhomoserine lactones (AHL). Degrading the AHLs can disrupt the Quorum Sensing (QS) modulated phenotypes such as virulence. Quorum quenching *Bacillus* sp. QSI-1 reduced pathogenicity of *A. hydrophila* in zebrafish (*Danio rerio*) and thereby improved the survival rate (Chu *et al.*, 2015). It will not necessarily decrease the pathogen load and an imbalance might still allow the opportunists to gain dominance.

While LAB strains seem somewhat promising as probiotics in aquaculture (Zorriehzahra *et al.*, 2016; Kuebutornye *et al.*, 2020), it is important to assess both strengths and weaknesses. If they are to be used as probiotics in larviculture they may not serve their full purpose in the early fish-life stages because the gastrointestinal tract is not fully developed and the microbiome inside the larvae is transient- being an extension of the microbiota in the tank (Bentzon-Tilia *et al.*, 2016)- which is not (yet) dominated by Firmicutes. Hence, other species that are adapted to and act in the marine environment are likely more suitable at this stage. Proteobacteria such as *Pseudomonas* spp., *Shewanella* spp., *Vibrio* spp., and members of the *Roseobacter* group have been proposed as non-LAB probiotics (Tapia-Paniagua *et al.*, 2014; Porsby and Gram, 2016; Dittmann *et al.*, 2017), especially due to their antagonism against pathogens. Gram *et al.* (2001) investigated the probiotic potential of *Pseudomonas fluorescens* strain AH2 against *V. anguillarum* and *A. salmonicida*. The growth of both pathogens was inhibited *in vitro* by *P. fluorescens* AH2 and the effect was increased during iron-limited growth conditions. This effect indicated that part of the probiotic effect could be due to iron competition (siderophores), although the experimental conditions did not allow for an exact determination of this

(Gram *et al.*, 1999). While the probiotic *Pseudomonas* could protect rainbow trout against vibriosis (Gram *et al.*, 1999), furunculosis caused by *A. salmonicida* in Atlantic salmon (*Salmo salar* L.) was unaffected by the probiotic treatment (Gram *et al.*, 2001). Hence, it is impossible to predict a “good” probiotic *insitu* based on *in vitro* experimental results; *in vivo* trials of probiotic candidates against different target pathogens in a variety of fish systems are required to determine their spectrum of activity.

Use of probiotic against pathogenic bacteria in fish aquaculture

The organisms that have the potential to improve the health or survival of farmed aquatic species are more diverse and greater in number than those used for human nutrition (i.e., mostly the lactic acid bacteria) and terrestrial veterinary medicine. Thus, a wide range of Gram-positive and Gram-negative bacteria, bacteriophages, unicellular algae, and yeasts as well as beneficial isolates from humans [*L. rhamnosus* (Nikoskelainen *et al.*, 2003) and *L. plantarum* (Picchietti *et al.*, 2007)] and terrestrial animals [*C. butyricum* from the intestine of healthy chickens (Pan *et al.*, 2008)] has been evaluated for use in aquaculture (Zorriehzahra *et al.*, 2016; Soliman *et al.*, 2019; Kuebutornye *et al.*, 2020; Ringø *et al.*, 2020; Zaczek *et al.*, 2020). This is because, the mode of action of probiotics is not similar and shows variations in efficacy, suggesting that one probiotic could not be used universally for all fish species to control single or multiple diseases. Among the Gram-positive bacteria, *Bacillus* species (such as *B. subtilis*) is the best evaluated genus for use in fish and shellfish farming, followed by various species of *Lactobacillus* (*L. plantarum*, *L. rhamnosus*). Other genera include *Arthrobacter*, *Brevibacillus*, *Brochothrix*, *Clostridium*, *Carnobacterium*, *Enterococcus*, *Kocuria*, *Lactococcus*, *Leuconostoc*, *Microbacterium*, *Micrococcus*, *Pediococcus*, *Rhodococcus*, *Streptococcus*, *Streptomyces*, *Vagococcus* and

Weissella. Although potential safety concerns due to the acquisition of virulence genes by horizontal gene transfer could be an issue in using Gram-negative bacteria, yet genera of fish or shellfish pathogens, such as *Aeromonas*, *Pseudomonas* and *Vibrio*, have been evaluated and found to be beneficial to aquatic hosts. Probiotics of these taxa include *A. hydrophila*, *A. sobria*, *P. fluorescens*, *P. chlororaphis*, *V. alginolyticus* and *V. proteolyticus*. Amongst other genera, *Agarivorans*, *Alteromonas*, *B. dellovibrio*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Neptunomonas*, *Phaeobacter*, *Pseudoalteromonas*, *Rhodo-pseudomonas*, *Roseobacter*, *Shewanella*, *Synechococcus*, *Thalassobacter* and *Zooshikella* have also demonstrated usefulness (Hai and Fotadar, 2009; Alagawany *et al.*, 2020 a,b,c; 2021). For example, dietary *Bacillus* and *A. sobria* were effective in controlling multiple bacteria, such as *V. anguillarum*, *V. ordalii*, *La. garvieae*, *A. salmonicida*, *S. iniae* and *Y. ruckeri*, in rainbow trout. Moreover, the efficacy of *Carnobacterium* sp. in reducing diseases caused by *A. salmonicida*, *V. ordalii* and *Y. ruckeri* in salmonids has been studied. Improved resistance to *V. anguillarum* in cod fry was also reported after the addition of *C. divergens* (Gildberg and Mikkelsen, 1998). The addition of strain JE-34 (*Zooshikella* sp.) in diets enhanced the innate immune response and disease resistance in olive flounder (*Paralichthys olivaceus*) against *S. iniae* (Kim *et al.*, 2010). When applied in water, a mixture of *L. plantarum*, *L. rhamnosus* and *L. salivarius* led to enhanced survival of *P. pelagicus* larvae (Talpur *et al.*, 2012). Moreover, *P. chlororaphis* induced disease resistance in perch against *A. sobria* (Gobeli *et al.*, 2009). In shellfish, *B. subtilis* E20 demonstrated effectiveness in controlling *V. alginolyticus* infection in white shrimp; probiotic supplements at concentrations of 10^6 , 10^7 , and 10^8 cfu/kg significantly increased survival rates by 13.3%, 16.7%, and 20% respectively, compared to the control group fed

no probiotic (Tseng *et al.*, 2009). Moreover, feeding with probiotic *Synechococcus* increased the survival of shrimp (*P. monodon*) post-larvae following a challenge with *V. harveyi* (Preetha *et al.*, 2007). Separately, *Pseudoalteromonas* protected scallop larvae against infection with *V. splendidus* and Pacific oyster against *V. coralliilyticus* (Kesarcodi-Watson *et al.*, 2012). *Arthrobacter* pXE-7 significantly enhanced the immune parameters in white shrimp and, at the same time, significantly reduced host mortality due to *V. parahaemolyticus* (Li *et al.*, 2012).

Bacteriophages from the viral families *Myoviridae* and *Podoviridae*, isolated from diseased ayu (*Plecoglossus altivelis*) and administered orally, have proved effective at reducing *P. plecoglossicida* infections in medicated fish and numbers of the pathogen in water (Park *et al.*, 2000). Moreover, administration of a lytic phage cocktail ϕ St2 and ϕ Grn1 (obtained from *V. alginolyticus*) directly to live prey artemia (*Artemia salina*) cultures led to a 93% decrease in presumptive *Vibrio* load after four hours of treatment, suggesting that the *Vibrio* population responsible for disease outbreaks in the marine larviculture system can be potentially eliminated by phage therapy (Kalatzis *et al.*, 2016). Microalgae such as *Dunaliella salina*, *D. tertiolecta*, *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Tetraselmis suecica* are also reported to promote growth, survival, and health of marine larvae (Supamattaya *et al.*, 2005; Marques *et al.*, 2006). Enhanced protection of gnotobiotic artemia against *V. campbellii* and *V. proteolyticus* was also conferred by the unicellular algal species *D. tertiolecta* (Marques *et al.*, 2006). The freshwater green microalga *Parietochloris incisa*, which is rich in arachidonic acid, was reported to have health benefits in the guppy (*Poecilia reticulata*) when administered as a dietary supplement for a period of 14 days (Dagar *et al.*, 2009).

The use of yeast holds great promise as a probiotic in aquaculture, yet this eukaryotic microbial group has received little attention. Different yeast products, such as cells and beta-glucan of *Saccharomyces cerevisiae*, *Phaffia rhodozyma*, and live *S. exiguus* containing xeaxanthin pigment (HPPR1), had a positive effect on juvenile *P. vannamei*, leading to better survival against *V. harveyi* (Scholz *et al.*, 1999). Oral administration of a polyamine (spermine and spermidine)-producing yeast, *Deba-ryomyces hansenii*, improved survivability but reduced the growth of European sea bass (*Dicentrarchus labrax*) larvae (Tovar *et al.*, 2002). A mixture of yeasts (isolates SS1, AY1) and bacteria (isolate SY9), which was added to dry feed at 10^7 cfu/g and fed to abalone (*Haliotis* spp.) for 14 days, led to 62% survival after challenge with *V. anguillarum* compared to 25% survival of the controls (Macey and Coyne, 2005). Also, marine yeast (*Yarrowia polytica*) supplementation (with microalgae) was effective in improving the survival and growth of juvenile pearl oyster, *Pinctada mazatlanica* (Aguilar-Macias *et al.*, 2010). Indeed, *S. cerevisiae* has been documented as a probiotic for catla (*Catla catla*) (Mohanty *et al.*, 1996), hybrid striped bass (*Morone chrysops* $\times$ *M. saxatilis*) (Li and Gatlin, 2004), flounder (Taoka *et al.*, 2006a), Nile tilapia (Taoka *et al.*, 2006b), and common carp (*Cyprinus carpio*) (Faramarzi *et al.*, 2011).

Conclusion

Probiotics as feed additives aid proper digestion of feed and make the nutrients available for faster growth and higher production. Immune functions can also be enhanced by the addition of probiotics to several infectious diseases of fish can be countered by adding probiotics to fish diets, but proper selection of the strain is needed for achieving optimal impact. Furthermore, probiotics can be used as beneficial bacteria to eliminate pathogens. Potentially, probiotics help to enhance nutrient digestibility, growth curve, productivity, feed efficiency and the health of fish. Future studies could generate more information and experience in order to understand probiotic sources and strains and their exact mode of action, as well as to explore their applications in fish production.

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