ISSN: 2456-2912
VET 2024; SP-9(1): 777-780
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www.veterinarypaper.com
Received: 15-10-2023
Accepted: 22-12-2023

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Comprehensive review of prebiotics and probiotics in aquaculture: Mechanisms and applications

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DOI: https://doi.org/10.22271/veterinary.2024.v9.i1Sk.1157

Abstract
Prebiotics and probiotics play crucial roles in aquaculture by promoting the growth and health of beneficial gut microbiota in aquatic species. Prebiotics, such as inulin and fructooligosaccharides, are non-digestible compounds that stimulate the growth of beneficial bacteria. On the other hand, probiotics, including various bacterial and yeast strains, directly contribute to intestinal microbial balance and host health. They act through mechanisms such as competitive exclusion, inhibitory compound production, immune response enhancement, and digestive enzyme production. While these interventions show Promise in improving aquaculture productivity and sustainability, further research is needed to fully understand their mechanisms of action and optimize their application.

Keywords: Comprehensive, prebiotics, Mechanisms, aquaculture, fructooligosaccharides

Introduction

Aquaculture, the farming of aquatic organisms, plays a crucial role in meeting the growing global demand for high quality protein. However, the industry faces challenges such as disease outbreaks, environmental impact, and the need for sustainable practices. In recent years, there has been increasing interest in using prebiotics and probiotics in aquaculture to improve production efficiency and sustainability.

Prebiotics are non-digestible compounds that stimulate the growth and activity of beneficial bacteria in the gastrointestinal tract, while probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts. Probiotics have been shown to enhance the immune response, improve growth performance, and increase disease resistance in various aquaculture species.

Despite the promising potential of prebiotics and probiotics in combination in aquaculture, their mechanisms of action and optimal application methods are still being studied. This article aims to review the current state of knowledge on prebiotics and probiotics in aquaculture, including their sources, mechanisms of action, and effects on aquatic species. It also discusses the criteria for selecting prebiotics and probiotics, as well as the concerns and challenges associated with their use in aquaculture.

Prebiotics

Prebiotics, in simpler terms, is any fiber, compound, nutrient, long-chain sugar, or substrate that is used as an energy source for the beneficial microorganisms (or probiotics) in the host digestive system (Panigrahi and Azad, 2007; Mountzouris, 2022; Wee *et al*., 2022) [17, 14, 24], the prebiotics does not get digested by the host because it is resistant to the acidic environment of host's stomach but it's able to stimulate the growth of a certain number of beneficial bacteria present in the gastrointestinal tract of the host organism (Gibson and Roberfroid 1995, Gupta 2011) [4, 6]. The host digestive system cannot digest prebiotics, but the beneficial gut microbiota can externally digest it by fermentation leading to the growth and multiplication of microbiota which leads to the improvement in host health. Inulin, arabinoxylan oligosaccharide, beta-glucan, mannan oligosaccharide (MOS), fructooligosaccharides (FOS), and oligosaccharides are some examples of prebiotics that are used very commonly in aquaculture (Wee *et al*., 2022) [24].

Several scientific reports prove that prebiotics can promote enhanced immune response, improved growth performance, increased efficiency of feed utilization, and better disease resistance of aquaculture species (Wee *et al*., 2022) [24]. By carefully understanding the research findings, it is obvious that there is a clear relation between the application of prebiotics and increased production of aquaculture species. Some prebiotics is specific to some gut microbes i.e., FOS and GOS, specifically found to promote the growth of Lactobacillus sp. and Bifidobacterium sp. as they have enzymes to fermentate FOS and GOS (Gibson *et al*., 2017; Teitelbaum and Walker, 2002) [5, 23]. Ideally, a mixture of prebiotics can be a good choice for obtaining a good yield in aquaculture. Microbial levan has also been claimed to have immunonutrient and prebiotic properties, but the feeding trials have been done only to a limited number of species; hence, further research is needed to be done to get reliable conclusions (Gupta *et al*., 2011) [6].

Probiotics

The term "probiotic" was coined by Parker in 1974, defining it as "*organisms and substances which contribute to intestinal microbial balance*." Fuller later revised the definition in 1989 to describe probiotics as a "live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance." Then, in 1992, Havenaar and Huis defined probiotics as "monocultures or mixed cultures of microorganisms applied to animals or humans, that benefit the host by improving properties of indigenous microflora" (FAO/WHO, 2001).

In 1998, Moriarty suggested expanding the definition of probiotics in aquaculture to include microbial "*water additives*." As a result, terms like "*good*," "*beneficial*," or "*healthy*" bacteria are commonly used interchangeably to characterize probiotics. While the application of probiotics in aquaculture appears relatively recent, the interest in environmentally friendly treatments has been rapidly growing. The increasing attention to probiotics in aquaculture is reflected in a growing number of studies, transitioning from empirical use to a more scientific approach. Consequently, there is now an opportunity to review the state of the art in probiotics, tracing its evolution from practical applications to a more comprehensive and research-driven understanding.

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus, B. subtilis, B. licheniformis, Nitrobacter sp*., *Aerobacter sp.*, and *Saccharomyces cerevisiae* while feed probiotics contain *Lactobacillus sp., Bacillus sp. or Saccharomyces cerevisiae* (Panigrahi and Azad, 2007) [17], examples include *Lactobacillus acidophilus* shown immune-boosting properties in Nile Tilapia by giving protection against *P. fluorescens* and *S. iniae* (Aly *et al.,* 2008) [1], *Bacillus subtilis* (controls *A. hydrophila* in *Labeo rohita* and increased immunity and resistance against *V. harveyi* in white shrimp, Kumar *et al.,* 2006, Zokaeifar *et al.*, 2012) [9, 28]. Powerful spore-forming bacteria acidify the stomach, inhibiting the growth of several pathogens such as *Salmonella, Clostridium, Streptococci, Escherichia,* and *Vibrio sp.* Together with the B group of vitamins, these bacteria can also release large amounts of enzymes, including lipase, protease, and amylase. This has a highly advantageous impact on feed material digestion. Several variables (poor diet, stress, antibiotics, aging) can tip the balance in favour of the pathogenic bacteria; in this case, probiotic bacteria might be helpful (Panigrahi and Azad, 2007) [17].

As compared to other probiotics, yeast-based probiotics have the unique advantage of producing polyamines, which have been observed to participate in numerous biological processes one such example of a probiotic is *Saccharomyces boulardii* a non-pathogenic yeast, which is considered a successful probiotic based on observable phenotypic features and physiological characteristics, such as optimum growth temperature, resilience to the gastrointestinal environment, and viability at low pH. Its ability to promote gut barrier integrity, exclude pathogens by competitive exclusion, produce antimicrobial peptides, modulate immunological response, and have trophic effects has all been linked to its probiotic action (Perdichizzi *et al*., 2023; Pais *et al*., 2020) [18, 15]. This yeast probiotic has been shown to have immunomodulatory action, and the possibility of anti-inflammatory action in the gut immune system of seabass (Perdichizzi *et al*., 2023) [18]. Savin *et al*., 2019 found that *Saccharomyces boulardii* has a positive effect on health, growth performance, and survival *rate on Cyprinus carpio* when supplemented with 2.5 percent of it. Sheikhzadeh *et al*., 2016 [22] concluded that *S. cerevisiae var. boulardii* strains fed *Onchorhynchus mykiss* have better disease resistance and survival as compared to *Onchorhynchus mykiss* fed with other strains of *S. cerevisiae.*

Streptomyces is a genus of actinobacteria with cosmopolitan distribution and the ability to produce novel bioactive compounds with antibiotic properties. As Actinobacteria are saprophytic and soil-dwelling they play a significant role in the turnover of complex biopolymers, like chitin, keratin, lignocellulose, hemicellulose (Williams *et al*., 1984) [25] and hence play a significant role in nutrient cycles and mineralization in aquaculture pond.

Marine actinomycete has been reported as a potential organism against the biofilm produced by *Vibrio spp.* and has also been recorded to act against diseases caused by Vibrio (You *et al*., 2007) [27].

Criteria for selection of probiotics

Selection of probiotics in aquaculture is based on limited scientific evidence (Pandiyan *et al*., 2013) [16]. In general, the criterion of selection depends upon bio-safety considerations

1. Production and processing techniques.
2. How the probiotic is administered.
3. Where in the body, the microorganisms are anticipated to be active (Huis *et al*., 1994).

Mechanisms of action of probiotics

Competitive exclusion of harmful (pathogenic) bacteria

In this, the probiotic bacteria compete for nutrition, sites of attachments in the mucosa, and produce inhibitory substances to hinder replication and/or destroy the pathogen, hence minimizing colonization (Moriarty, 1998) [13].

Inhibitory compound production

Antagonism is a common kind of bacterial interaction in the environment. Chemicals produced by microorganisms, in this case, bacteria that are toxic (bactericidal) or inhibitory (bacteriostatic) towards other microorganisms are known as antagonistic chemicals. It is believed that the presence of bacteria that produce antibacterial substances in the host's intestine, on its surface, or in its culture water inhibits the growth of harmful microbes and may even eradicate them (Pandiyan *et al*., 2013) [16]. According to Yang *et al*., (2010) [26] two LAB strains (Lactococcus lactis MM1 and MM4) have the ability to release chemicals similar to bacteriocin and hydrogen peroxide, which have potent inhibitory effects on pathogens such *V. metschnikovi*, *V. harveyi*, and *S. aureus*, which infects orange-spotted grouper. Isolated lipopetides N3, which were generated by *B. amyloliquefaciens* M1 and significantly inhibited the growth of bacteria on both the cell membrane and the entire cell. In addition, lipopeptide N3's antibacterial activity may result from its capacity to create ion-conducting channels in bacterial cell membranes by taking advantage of its detergent-like action on cell membranes, a property known as membrane-active characteristics.

Enhancing the immune response of the host

Some of the aquatic animals cultured have innate or non-specific immunity (e.g., invertebrates like shrimp, crab, etc.), while some have both non-specific and specific/acquired/adaptive immunity (e.g., vertebrates like fish). The advantage of using probiotics is that it stimulates the innate immune system, which is common in both fish and shellfish. The innate immune system is comprised of humoral and cellular components along with physical barriers. While nonspecific cytotoxic cells and phagocytes make up innate cellular immune effectors, innate humoral parameters include antimicrobial peptides, lysozyme, complement components, transferring, pentraxins, lectins, antiproteases, and natural antibodies (Pandiyan *et al*., 2013) [16]. It has been shown that giving rainbow trout oral administration of *Clostridium butyricum* bacteria increased the fish's resistance to vibriosis by enhancing leucocyte phagocytic activity (Pandiyan *et al*., 2013) [16]. According to Rengpipat *et al*. (2000) [20], tiger prawns (*Penaeus monodon*) were protected against disease through the activation of both cellular and humoral immune defenses when Bacillus sp. (strain S11) was used. Balcazar (2003) [2] provided evidence that the inclusion of a combination of Bacillus and Vibrio sp. bacterial strains in feed improved the growth and survival of juvenile white shrimp and enhanced the non-specific immune system by boosting phagocytosis and antibacterial activity. According to a recent study by Qin *et al*. (2017) [19], *L. casei* BL23 protected zebrafish larvae from infection by *A. veronii*. Additional information suggested that *L. casei* BL23 may have improved host immune responses through the TLR1/TLR2 pathways through the exopolysaccharide-protein complex (EPSP) from BL23.

Production of digestive enzymes

It has been observed that growth performance, feed digestion, and absorption is enhanced by the higher enzymatic activities of the digestive system. In probiotic-fed silver pomfret (*Pampas argenteus*), Gao *et al*. (2016) [3] showed a probable association between increased growth rate and increased intestinal digesting enzyme activity. Several *Bacillus species* possessing exo-enzyme activities have been demonstrated in earlier research to considerably enhance the host's growth performance (Liu *et al*., 2017; Han *et al*., 2015; Liu *et al*., 2009, Liu *et al*., 2012) [12, 7, 11, 10]. This improvement may be linked to the production of digesting enzymes.

Concerns

Certain bacterial cultures obviously help the host grow more rapidly and experience fewer health problems. A decrease in the usage of antibiotics could be one indirect advantage. Probiotics' exact mode of action is, not properly understood. It is important to make sure the probiotic is safe for the host, thus care must be taken while selecting one. Probiotics from species thought to be harmful to aquatic animals (*V. alginolyticus* is one example) should be avoided, as should the absence of suitable *in vivo* challenge experiments (Irianto *et al*., 2002) [8]. It worries that organisms that at first glance appear to be harmless could revert to their virulence or spread to other species.

Conclusion

In conclusion, prebiotics and probiotics offer significant potential in enhancing aquaculture production and sustainability. Their ability to improve immune responses, growth performance, and disease resistance in aquatic species makes them valuable tools for aquaculturists. However, careful selection of prebiotics and probiotics based on scientific evidence and bio-safety considerations is essential to ensure their effectiveness and safety. Before fully relying on prebiotics and post biotics it is needed to optimize their formulations, dosages, and delivery methods for different fish species and production systems. Further research is needed to explore the full range of benefits and mechanisms of action of these interventions, as well as their potential impact on aquatic ecosystems.

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