Research Article

Potential of Probiotics in Sustainable Aquaculture Production in Pakistan

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Abstract |Probiotics may affect the gut microbiota of many different kinds of animals, including aquatic organisms. Probiotics are well known for reduction the risk of illness and stress, boosting immunity, altering gut microbiota, digestion, and nutrition, as well as improving the quality of water. In addition to the aforementioned, they improve the feed conversion, stimulate growth, and increase the rate of reproducing and hatching in pond systems. This document covers a brief review of the importance of probiotics in marine sciences that studied so far focusing on Pakistani aquaculture. Sustainable aquaculture production holds significant potential for probiotics in Pakistan by enhancing fish health, improving water quality, and reducing the need for antibiotics, thereby promoting eco-friendly practices. To prevent and treat infections, as well as improve the quality and sustainability of aquaculture output, it is important to create an antibiotic-free alternative to combat the ongoing growth of antibiotic-resistant bacteria as a result of antibiotic use in aquaculture. The prevalence of disease in aquaculture, combined with the need for aquaculture sustainability, encouraged researchers to study and the use of probiotics on aquatic animal species. The use of probiotics was limited to stimulating development and increasing the production of the aquatic organisms however, their effect on stress tolerance and reproduction has been explored late, but still there is a need for more scientific research in these areas. Probiotics can be used in various ways but the incorporation of production as a plant based supplemented diets, addition in water and directly injected to the fish can boost fish growth, immunity and disease resistance which are important for country's aquaculture sector. High quality probiotic product insufficient research on region-specific strains and lack of awareness among farmers are the limitations in Pakistan for the adoption of probiotics. The infrastructure and the regulatory framework for the maximum distribution and production of probiotics are underdevelopment which hinders the application and effectiveness of probiotics.

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Introduction

Aquaculture is the cultivation of aquatic organisms in both coastal and inland which includes interventions in the growing process to maximize productivity (FAO, 2020). Fisheries science and aquaculture management are fundamentally guided by key biological parameters such as fish growth, body composition, and reproductive performance, which are critical for achieving sustainable production and maintaining ecological balance. A comprehensive understanding of these traits is essential for evaluating fish health, refining breeding programs, and enhancing aquaculture productivity. Among these, the weight-length relationship and condition factor serve as widely recognized indicators of growth efficiency and physiological well-being in fish populations, thereby facilitating robust stock assessment and effective management strategies (Javed *et al*., 1992; Naeem *et al*., 2005a; Naeem *et al*., 2010a; Naeem *et al*., 2011a). Furthermore, the proximate and elemental composition of fish, which varies with body size and physiological condition, significantly influences nutritional quality and market value (Naeem *et al*., 2010b; Ashraf *et al*., 2011; Yousaf *et al*., 2011; Naeem *et al*., 2016; Naeem *et al*., 2017). Advancements in reproductive biology, particularly in induced breeding techniques, have contributed to improved spawning outcomes and a steady supply of economically valuable species. Hormonal treatments such as Ovaprim-C have demonstrated efficacy in enhancing fecundity, fertilization, and hatching success, thereby supporting hatchery-based propagation (Naeem *et al*., 2005b; Naeem *et al*., 2011b). Additionally, dietary modulation plays a pivotal role in influencing growth performance, feed conversion efficiency, and the overall sustainability of aquaculture systems, with tailored nutritional formulations shown to significantly enhance fish health and development (Ismat *et al*., 2013).

One of the major problem with aquaculture expansion has been the spread of diseases. Chemical agents and antibiotics have historically been the most common methods of infection control. (Assefa and Abunna, 2018). The use of antibiotic treatments has been limited in many countries due to the bacterial antibiotic resistance and bioaccumulation affect having the negative consequences on both human and animal health (Rodgers and Furons, 2009; Santos and Ramos, 2018). The use of the probiotics and the vaccinations are the two alternative ways to treat with the infections against the viral, microbial and bacterial infections. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have defined that the live microorganisms which are administrated in the proper dose and have the health benefits in the host are called as probiotics (Hill *et al*., 2014). In aquaculture, the probiotics can be considered as an alive component of microbial cell which administered via feed, injected or added into the water which benefits the host by improving the growth, disease resistance, feed utilization, stress response and growth performance. Probiotics primarily promote disease resistance by immunological parameter modulation, competition for binding sites, antibacterial substance synthesis, and nutrition competition. In the recent years, outstanding reviews were published by using the probiotics in the aquaculture (Zorriehzahra *et al*., 2016; Hoseinifar *et al*., 2018). They reported that the role of probiotic microorganisms and their effects in aquaculture played vital role in the disease resistance and disease control in the aquaculture, while recombinant probiotics function as antibiotic treatment as well alternative or complementary treatment against disease. To summarize, the vital role of probiotics has also important in innate immunity, micronutrient absorption and the macronutrient absorption. Furthermore, probiotics can interact with immune cells in fish's GITs, activating immunological responses that promote fish development and protect against pathogen infection. Symbiotic method is likely to become a popular method for preventing and controlling bacterial infections in fish farms (Sewaka *et al*., 2019)

Utilization of probiotics in aquaculture

Since Fleming discovered penicillin in 1928, antibiotics have been used to treat infections and diseases in both people and animals (Fleming, 1944). Antibiotics are also good to animals and aquaculture, both as a prophylactic strategy and to boost growth (Marshall and Levy, 2011; Tan *et al*., 2016). Antibiotics are used in aquaculture to promote rapid and efficient growth. The researchers indicated that antibiotic resistance genes exist in aquaculture ponds (Tomova *et al*., 2015; Xiong *et al*., 2015). A gene transfer approach allows the animal and human illness to acquire antibiotic resistance (Tomova *et al*., 2015). The treatment of bacteria-related disorders and recent data suggests that leftover antibiotics in raising animals may offer health hazards to humans (Chen *et al*., 2015; Pereira *et al*., 2015).

Role of probiotics in growth and survival rate

Probiotics have been incorporated in aquaculture to stimulate the growth of various cultivable species. Allameh *et al*. (2016) added *Enterococcus faecalis* species having 10^7 and 10^9 cfu/g to the feed of Javanese stock

(*Puntius gonionotus*), they observed the substantial increase in its weight. These microorganisms reside in the gut and due to their higher growth rate their population dominate very shortly suppressing the role of harmful bio-fauna. As they strengthen the immunity of the host, maintains its health and sustains the metabolism and provision of required nutrients hence their persistent input is mandatory to dominate their population and outweigh the harmful bacterial fauna (Banerjee and Ray, 2017). Probiotics promote host growth by increasing feed intake and nutrient absorption (Nath *et al*., 2019; Sharma *et al*., 2013). This increases both survival rates and growth performance while utilizing probiotics. Lactobacillus is the most thoroughly researched bacteria for its effect on growth. A meal containing *L. plantarum* increased growth in a variety of fish species, including carp, Nile tilapia, brown trout, and Salmo trutta caspius (Dawood *et al*., 2019). In addition, combining *L. plantarum* with natural immune stimulants and probiotics boosts growth rates. Hamdan *et al*. (2016) found that feeding Nile tilapia (*Oreochromis niloticus*) with *Lactobacillus sp.* supplemented feed resulted in higher crude lipid (CP) and total protein (TP) availability for better growth. Water quality, diversity of hydrobiont species, differing enzyme levels, and the organism's genetic resistance all have an impact on the success of probiotic treatments. Tan *et al*. (2016) had documented that *Xiphophorus helleri, Xiphophorus maculates,* and *Poecilia reticulate* grew and survived better when administered probiotics comprising *Bacillus subtilis* and *Streptomyces sp*.

Role of probiotics in nutrient utilization

Probiotics facilitate nutrient digestion in fresh/sea water animals and produce energy and other products required for growth. Lactic acid bacteria and their products are frequently used as growth enhancers in commercially important fish species (Ringo *et al*., 2018). The Food and Drug Administration (FDA) has declared that they are safe for animal and human usage and utilization because of their natural and benign presence facilitating the health and well-being of these organisms (Giri *et al*., 2013).

Probiotic supplemented feed enhanced the existing levels cellulases, amylases, and proteases. Similar impact has been observed on phytases produced due to the probiotic microorganisms in the host gut (Burr and Gatlin, 2005; Banerjee *et al*., 2017; Ghosh *et al*., 2017). As mentioned earlier some has the capability to increase the level of digestive enzymes but also improve their absorptive capability facilitating efficient utilization of the feed taken in. A Study of Wang and Wang (2008) support it as well as previous claims when he observed higher activities of proteases, lipases, amylases, and cellulases enzymes in shrimps, *Litopenaeus vannamei*, and bivalves, when fed on *Rhodobacter sphaeroides* and *Bacillus sp* supplemented diets. In addition to the above actions, Probiotics may also increase the surface area of the host GIT by causing histological changes in the morphology of intestinal folds including enterochromaffin cells, and microvilli (Zhou *et al*., 2010).

Priyodip *et al*. (2017) in their study observed production of large quantities of phytase due the inclusion of *Lactobacillus brevis* and *Bacillus subtilis* in the diet. This enzyme aids in the utilization and metabolism of the plant products such as phytate (myo-inositol hexaphosphate). Ringo *et al*. (2014) in their study reported the positive impact of *Pseudomonas sp., Brevibacterium sp., Microbacterium sp., Agrobacterium sp.*, and *Staphylococcus sp.,* on the nutritional status and physiology of the Arctic charr (*Salvelinus alpines*). Moreover, some bacterial probiotics synthesize vital amino acids, minerals, and fatty acids and alter the microbial population of the host gut (Nayak, 2010; Newaj-Fyzul *et al*., 2014).

Probiotics mode of action in aquaculture

When antibiotics are continuously used to treat the diseases in aquaculture species, ultimately generation of resistance genes, and tolerant bacterial fauna develops. Subsequently bacterial population proliferates and drug residue builds up in fish tissues due to their poor utilization. Applications of vaccines for framed fish are currently used though still at limited scale. For extraction of maximum benefits their proper and timely usage is of paramount importance otherwise their effectiveness declines swiftly. Among others their adequate and efficient delivery is significant. Due to their disease management qualities probiotics are considered pre-requisite for sustainable aquaculture. Irianto and Austin (2002) have reported that probiotics improves water quality, and boost host immunity against the potential diseases resulting in better fish survival.

Effect on phytoplankton

Probiotic regulates and manage the production of algae, especially red tide planktons at safe level (Qi *et* al., 2009). For early fish fry rearing sufficient quantity of planktons are required to be present in ponds. However, harmful bacteria can limit or totally can wipeout their production unicellular algae in specific. Nonetheless probiotics can solve this issue and help for the, proliferation of phytoplankton.

Bacteriostatic effect of probiotics

Probiotics release many bacteriostatic or bactericidal chemicals which are equally effective for the control of both Gram-positive and Gram-negative bacteria. Hydrogen peroxides, proteinaceous enzymes (lysozyme and other proteases), and iron-chelating (sideropheres) compounds are the most common ones (Giri *et al*., 2013). Sometimes probiotics produce bacteriocins which change chemicals or energy preferences of the gut fauna and break inter and intra species interactions and ultimately overcoming their excessive population pathogens, their interruptions and effects (Kesarcodi-Watson *et al*., 2008; Ringo *et al*., 2018).

Mode of action in controlling infections

Probiotics strengthens defensive mechanism in the gut of the host limiting the level of infections following inflammations (Azimirad *et al*., 2016; Modanloo *et al*., 2017). Probiotic microorganisms such as lactic acid bacteria, *Brevibacillus brevis*, *Vagococcus fluvialis*, and *Vibrio harveyi*, adhere to the mucosal epithelium of the gastrointestinal tract, modulate immunity of the host, and prevent attachment and pathogenicity of the potential pathogens (Lazado *et al*., 2011; Sugimura *et al*., 2011; Korkea-aho *et al*., 2012; Mahdhi *et al*., 2012; Sorroza *et al*., 2012). Production of enzymes such as proteases, amylases and alginate lyases are further strengthen this mechanism and manage the uncontrolled proliferation of pathogens (Zokaeifar *et al*., 2012). Production of essential nutrients like fatty acids, biotin, vitamin B_{12}, and organic acids during this cascade of activities are further ameliorates this process.

Competition for nutrients

during infectious stage harmful and useful bacteria compete for nutrient uptake. probiotics disrupt this competition and tilt the balance towards useful microbial population strengthening their potential capability (ringo *et al*., 2016). it has been well documented in marine bacteria where they compete for uptake of iron (verschuere *et al*., 2000). however, the amount of iron present in animal tissues and body fluids is limited (verschuere *et al*., 2000). the iron-binding substances called siderophores assist bacteria in obtaining the iron they require for development.

possible beneficial effects of some specific probiotics and their particular applications have been given in the table 1.

Table 1: *Some probiotics documented for the promotion of aquaculture*

Probiotic	Application	Applied to aquatic species	Described/ Expected outcome
Lactobacillus helveticus (Gatesoupe, 1999)	Growth promoter	*Scophthalmus maximus*	Affects Gut movement and helps in digestion which also leads to energy production.
Bacillus coagulans *(Lin et al. 2012)*	Growth promoter	*Cyprinus carpio* koi	Increases the level of digestive enzymes hence increases digestion.
Pseudomonas fluorescens *(Gram et al. 1999)*	Pathogen inhibition	*Oncorhynchus mykiss*	Acts as antibiotic and may replace vaccines. Replacing vaccines is necessary as it causes stress when administered.
L. rhamnosus ATCC53103 (Nikoskelainen *et al*. 2001)	Pathogen inhibition	*Oncorhynchus mykiss*	It contains inhibitory substances which acts as pathogen inhibitor.
Lactobacillus aciophilus (Dohail *et al*. 2009)	Nutrient digestibility	*Clarias gariepinus*	It produces organic acids and other compounds which have positive effects on host health and its growth.
L. helveticus (Gatesoupe, 1999)	Nutrient digestibility	*Scophthalmus maximus*	Helps in conversion of organic materials into CO_2 and maintains a balance in phytoplankton production.
Bacillus NL 110, *Vibrio* sp. NE 17 (Rahiman *et al*. 2010)	Water quality	*Macrobrachium rosenbergii*	It is also applicable in other industries such as dairy and makes the foods as functional foods.
Bacillus subtilis (Ghosh *et al*. 2007)	Reproduction improvement	*Poecilia reticulata*, *Xiphophorus maculatus*	May restore and improve the quality of fish sperm exposed to heavy metals.

There is direct correlation between infectious pathogenicity and siderophore synthesis in the animal body (gram *et al*., 1999). probiotics produce siderophores which sequester the available iron making it less available for pathogens endangering their survival. Probiotics have also been shown to improve feed utilization and growth performance in aquatic animals by raising the levels of digestive enzymes (yu *et al*., 2009; zokaeifar *et al*., 2012; hoseinifar *et al*., 2017). it has been documented that the growth performance of western king prawns was improved by dietary probiotics (*pseudomonas aeruginosa* and *ps. synxantha*) (van-hai *et al*., 2009; hai *et al*., 2010). extracellular enzymes such as proteases, carbohydrolases, and lipases have been reported to increase the digestive enzymatic activities and an improvement in the digestive process after probiotic treatment (arellano-carbajal and olmos-soto, 2002; leonel and olmos-soto, 2006; soleimani *et al*., 2012; eshaghzadeh *et al*., 2015; hoseinifar *et al*., 2015a, b). moreover, crucial nutrients such as fatty acids, biotin and vitamins, and probiotics might be a complementary food source to sustain health as well as promote growth of cultured animals.

Conclusions

The use of probiotics helps in promoting fish health, improving water quality, boosting growth rate and enhancing aquaculture practices in Pakistan. Probiotics are also helping in reducing the need of antibiotics, balancing microbial environment and enhancing immune response of aquatic species. The use of Probiotic in Pakistan has shown sustainable aquaculture development and increasing productivity while minimizing environmental impact.

Probiotics have considerable and promising feature for increasing the sustainability and environmental friendliness of applications of expensive chemotherapeutic drugs for treatment exert negative impacts not only on the water quality but deteriorates the meat quality of fish transporting some undesirable's medicinal residues to the consumers. Probiotics are therefore a promising alternative approach for controlling the infectious agents as well as disease they cause to the aquatic population.

Recommendations

Aquaculture is an important food producing sector to satisfy nutritional demands of the growing human populations. However, incapacitated feed utilization and unexpected disease outbreak has considerably affected fish production negatively and a reason for heavy economic losses to aquaculture industry. Feed additives must be used in innovative ways to boost the immune systems, disease resistance, and growth performance of aquaculture species. In the context of rapidly increasing global aquaculture production, it is important to find alternative environmentally friendly approaches for illness prevention and treatment, as well as to improve growth performance. However, further research is required to fully understand the mechanisms of probiotic action and their environmental impact. For example, the influence of probiotics on the aquatic microbial population is not well understood, and further research is needed into potential ecological hazards associated with probiotic use in aquaculture. Furthermore, the efficacy of probiotics can be influenced by a number of factors, including the probiotic strain utilized, the dose, and the method of delivery, necessitating future research to optimize their application. Before being developed into fish probiotics, candidate strains must be subjected to rigorous evaluation under a global standard. So far, they have proved themselves very effective but still a lot more needs to be done until the establishment of their successful and long lasting impact on the overall production and promotion of aquaculture sector. This review is a humble attempt to briefly highlight the role of probiotics in aquaculture. Maintenance of its permanent culture at aquaculture facility can be a cumbersome for new entrants in this field but it is priority to obviate any set back during this whole process. Time may come in near future, when instead of ordering antibiotics and feed additives, specific probiotics will be called upon for provision of quality. Various fisheries research units and the fisheries departments of different universities in a country should focus on research and on the role of probiotic bacterial species from different natural habitats as well as culture ponds. Researches indicated that the use of probiotic in early age of fish growth have the benefits such as better feed conversion ratio, improved disease resistance and reduced the risk of fish mortality. The widespread uses of probiotics and awareness among farmer regarding the safe and potential uses of probiotics are needed. If these challenges are addressed then it may be possible the potential role of probiotics in advancing sustainable aquaculture by contributing of food security, economic growth and environmentally friendly aquaculture practices increase with increased export potential. Applications of different probiotics species then be assessed under varying conditions of geographical locations, fish species and infectious disease outbreaks for recognizing particular probiotic species for particular application to obtain healthy yield in ecofriendly environment. This identification, isolation, and further extension can be salvation to the future probiotic availability, storage and utilization problems, and limitations.

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Novelty Statement

The manuscript provides an insight for the development and progress of aquaculture and sustainable aquaculture farming in Pakistan.

Author's Contribution

Sadaf Aman: Writing - original draft, writing - review & editing
Sajid Mahmood Shahzad: Resources, supervision
Rahman Ullah: Data curation, software

Generative AI and AI-assisted technology statement
The authors declare that no Generative AI was used in the creation of this manuscript.

Conflict of interest
The authors have no conflict of interest.

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