

Review Article

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Role of Probiotics on Aquaculture: Importance and Future Perspectives

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ABSTRACT

Aquaculture is one of the fastest developing growth sectors in the world and Asia presently contributes about 90% to the global production. But disease outbreaks are constraint to aquaculture production thereby affects both economic development of the country and socio-economic status of the local people in many countries of Asia-Pacific region. Disease control in aquaculture industry has been achieved by following different methods using traditional ways, synthetic chemicals and antibiotics. The use of such expensive chemotherapeutants for controlling diseases has been widely criticized for their negative impacts like accumulation of residues, development of drug resistance, immunosuppressant's and reduced consumer preference for aqua products treated with antibiotics and traditional methods are ineffective against controlling new diseases in large scale aquaculture systems. Therefore, alternative methods need to be developed to maintain a healthy microbial environment in the aquaculture systems there by to maintain the health of the cultured organisms. Use of probiotics is one of such method that is gaining importance in controlling potential pathogens. This review provides a summary of the criteria for the selection of the potential probiotics, their importance and future perspectives in aquaculture industry.

Keywords

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Introduction

Aquaculture is the farming of aquatic organisms by intervention in the rearing process to enhance production and private ownership of the stock being cultivated. Compared to fishing, this activity allows a selective increase in the production of species used for human consumption, industry or sport fishing.

Due to overfishing of wild populations, aquaculture has become an economic activity of great importance around the world. Aquaculture's contribution to world food

production, raw materials for industrial and pharmaceutical use, and aquatic organisms for stocking or ornamental trade has increased dramatically in recent decades.

Aquaculture has a long history, originating at least in the year 475 B.C. in China (Timmons *et al.*, 2002), but became important in the late nineteen-forties, since the methods of aquaculture could be used to restock the waters as a complement to natural spawning. Nowadays, aquaculture is a lucrative industry (Boyd and Tucker, 1998; Cressey, 2009). The intensification of aquaculture

practices requires cultivation at high densities, which has caused significant damage to the environment due to discharges of concentrated organic wastes, that deplete dissolved oxygen in ponds, gives rise to toxic metabolites (such as hydrogen sulfide, methane, ammonia, and nitrites), that often are responsible for mortality. Additionally, aquaculture has appropriated of water bodies used for recreational purpose, and sometimes makes water's waste because this natural resource is not reused in extensive aquaculture systems (Amaya and Castellano, 2006; Wang and Xu, 2004).

In large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Prevention and control of diseases have led during recent decades to a substantial increase in the use of veterinary medicines. However, the utility of antimicrobial agents as a preventive measure has been questioned, given the extensive documentation of the evolution of antimicrobial resistance among pathogenic bacteria (Balacazar, 2003). Globally, tones of antibiotics have been distributed in the biosphere during an antibiotic era of only about 60 years duration. In the United States, out of the 18,000 tons of antibiotics produced each year for medical and agricultural purposes, 12,600 tonnes are used for the non-therapeutic treatments of livestock in order to promote growth SCAN (2003).

In the European Union and Switzerland, 1600tons of antibiotics, representing about 30% of the total use of antibiotics in farm animals, are similarly used for growth promotion purposes. These amounts of antibiotics have exerted a very strong selection pressure towards resistance among bacteria, which have adapted to this situation, mainly by a horizontal and promiscuous flow of resistance genes (SCAN, 2003; Sahu *et al.*, 2008). Resistance mechanisms can arise one of two ways: chromosomal mutation or acquisition of plasmids. Chromosomal mutations cannot be transferred to other bacteria but plasmids can transfer resistance rapidly (Lewin, 1992). Several bacterial pathogens can develop plasmid-mediated resistance.

Therefore, alternative methods need to be developed to maintain a healthy microbial environment in the aquaculture systems there by to maintain the health of the cultured organisms. Use of probiotics is one of such method that is gaining importance in controlling potential

pathogens. This review provides a summary of the criteria for the selection of the potential probiotics, their importance and future perspectives in aquaculture industry.

Definition of Probiotic

The term "probiotic" comes from Greek word *pro* and *bios* meaning "prolife" (Schrezenmeir and De verse, 2001), having different meanings over the years. In 1905, Dr. Elie Metchnikoff was the first to describe the positive role played by some bacteria among farmers who consumed pathogen-containing milk and that "reliance on gut microbes for food makes it possible to take steps to change the flora of our bodies and to replace harmful microbes by beneficial microbes" Metchnikoff (1907).

However, the term probiotic was introduced until 1965 by Lilly and Stillwell (1965) as a modification of the original word "probiotika." It was used to describe substances produced by a microorganism that prolong the logarithmic growth phase in other species. It was described as an agent who has the opposite function of antibiotics. Later, Sperti (1971) modified the concept of "tissue extracts that stimulate microbial growth."

The first use of the term to describe a microbial feed/food supplement was by Parker (1974). He defined it as "organisms and substances that contribute to intestinal microbial balance." Fuller (1998) expanded the definition to "live microbial food supplement that benefits the host (human or animal) by improving the microbial balance of the body" and said that it would be effective in a range of extreme temperatures and salinity variations.

Knowledge of probiotics has increased; currently it is known that these microorganisms have an antimicrobial effect through modifying the intestinal microbiota, secreting antibacterial substances (bacteriocins and organic acids), competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect. Probiotics are also capable of modulating the immune system, regulating allergic response of the body, and reducing proliferation of cancer in mammals. Because of this, when provided at certain concentration and viability, probiotics favourably affect host health (Myers, 2007). In fact, terms such as "friendly bacteria," "friendly," or "healthy" are commonly used to describe probiotics (Wang *et al.*, 2008).

Probiotics in Aquaculture

Extended definition

When looking at probiotics intended for an aquatic usage it is important to consider certain influencing factors that are fundamentally different from terrestrial based probiotics. Aquatic animals have a much closer relationship with their external environment. Potential pathogens are able to maintain themselves in the external environment of the animal (water) and proliferate independently of the host animal (Hansen and Olafsen, 1999; Verschuere *et al.*, 2000a).

These potential pathogens are taken up constantly by the animal through the processes of osmoregulation and feeding. A study with Atlantic halibut, *Hippoglossus hippoglossus*, showed the transition from a prevailing *Flavobacterium spp.* intestinal flora to an *Aeromonas spp.*, *Vibrio spp.* dominant flora occurred when first feeding commenced (Bergh *et al.*, 1994).

Based on the intricate relationship an aquatic organism has with the external environment when compared with that of terrestrial animals, the definition of a probiotic for aquatic environments needs to be modified. Verschuere *et al.*, (2000a) suggested the definition “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”. Apart from the requirement of the probiotic to be a live culture, this definition is a lengthy way of describing a probiotic as defined by Irian to and Austin (2002a) thus “a probiotic is an entire or components of a microorganism that is beneficial to the health of the host”.

Commercial Probiotics Preparations

The interest in probiotics as an environmentally friendly alternative is increasing and its application is both empirical and scientific. According to Socol *et al.*, (2010), the global market for probiotic ingredients, supplements and foods, reached US \$15,900 million in 2008 and is projected to increase to US \$19,600 million in 2013, representing an annual growth rate of 4.3%. At present, there are several commercial preparations of probiotics that contain one or more live microorganisms, which have been introduced to improve the cultivation of

aquatic organisms. Probiotics can be used as a food additive added directly to the culture tank or mixed with food.

Apart from laboratory preparation of bacteria, some commercially available products are now available. One of the first evaluations of commercial products focussed on a bacterial preparation called Bio-start that is derived from *Bacillus* isolates. It was used during the production of cultured catfish studying the effect of inoculum concentration (Queiroz & Boyd, 1998).

In 1998, Moriarty reported that the use of commercial probiotic strains of *Bacillus spp.* increased the quality and viability of pond-raised shrimp.

The lactic acid-producing bacteria have been the focus of much interest. The human probiotic, *Lactobacillus rhamnosus* ATCC (American Type Culture Collection, Rockville, MD, USA), was used in rainbow trout for 51 days to reduce mortality by *Aeromonas salmonicida*, the causative agent of the fish disease “furunculosis” (one of the major fish diseases in many parts of world).

Mortality was reduced from 52.6 to 18.9% when 109 cells g⁻¹ were administered with feed, when probiotic dose was increased to 1012 cells g⁻¹ of feed the mortality reached 46.3% (Nikoskelainen *et al.*, 2001). Apparently, increasing dosage does not necessarily improve protection. Abasali and Mohamad (2010) increased the gonadosomatic index and the production of fingerlings in females of reproductive age, using mixed cultures consisting of *L. acidophilus*, *L. casei*, *E. faecium*, and *B. thermophilum* (Primalac).

Commercial preparations with live lactic acid bacteria have also been introduced into the medium of live food organisms for larval flatfish. Some of these treatments increased the production of rotifers and the growth of turbot and Japanese flounder (Gatesoupe, 1989, 1991; Gatesoupe *et al.*, 1993). Some preparations with lactic acid bacteria limited also the proliferation of bacteria in rotifers, but the fate of the lactic acid bacteria was not studied in these experiments (Gatesoupe *et al.*, 1993; Gatesoupe, 1991).

Other commercial preparations of *Streptococcus faecium* improved the growth and feed efficiency of Ivanova *et al.*, (1998); Bogut *et al.*, (1998). *Escherichia coli* disappeared from the intestinal microbiota of carp after 14 days of feeding with the probiotic preparation Bogut

et al., (1998). These authors stated that *S. faecium* “has high adhesive ability in the epithelium of carp digestive tract”, but without any experimental evidence.

Meanwhile, Taoka *et al.*, (2006) used Alchem Poseidon and Alchem Korea CO and Wonju Korea CO, which have mixed cultures of bacteria (*Bacillus subtilis*, *Lactobacillus acidophilus*, and *Clostridium butyricum*) and yeast (*Saccharomyces cerevisiae*), enhanced nonspecific immune parameters of tilapia *Oreochromis niloticus* such as lysozyme activity, migration on neutrophils, and plasma bactericidal activity, resulting in improvement of resistance to *Edwardsiella tarda* infection. Previous studies in humans and land animals using prebiotics (no digestible ingredients of the diet that stimulates the growth of microorganisms) showed their ability to stimulate the activity of probiotic bacteria in the colon (Martinez *et al.*, 2008; Tuohy *et al.*, 2003).

Currently, commercial products are available in liquid or powder presentations, and various technologies have been developed for improvement on the case of fermentation processes, the interest has been focused on optimizing the fermentation conditions to increase the viability and functionality of probiotics, improving performance (Lacroix & Yildirim, 2007). Generally, the production is carried out in batch cultures due to the difficulty of industrial scale operation of continuous systems (Soccol *et al.*, 2010). More recently, systems have been developed for immobilization of probiotics, especially using microencapsulation.

The methods commonly used for microencapsulation of probiotics are the emulsion, extrusion, spray drying, and adhesion to starch (Rokka & Rantam, 2010). Focused on the application to aquaculture, Rosas *et al.*, (2012) have effectively encapsulated cells of *Shewanella putrefaciens* in calcium alginate, demonstrating the survival of encapsulated probiotic cells through the gastrointestinal tract of sole (*Solea senegalensis*). Encapsulation in alginate matrices protects bacteria from low pH and digestive enzymes; this protection helps to release the probiotic into the intestine without any significant damage (Morinio *et al.*, 2008).

Currently, the lyophilized commercial preparations have advantages for storage and transport. However, conditions for reconstitution of these preparations such as temperature, degree of hydration, and osmolality of the solution are vital to ensure the viability of bacteria (Muller *et al.*, 2008). It is important to emphasize that

these products must provide a health benefit to the host; for this, it is necessary that contained microorganisms have the ability to survive storage conditions, and after that in the digestive tract of aquatic species, remaining viable and stable, and finally improving production (Irianto & Austin, 2002). According to the opinion of the producers, these preparations are safe to use and effective in preserving the health of aquatic animals (Wang *et al.*, 2010).

Search for Autochthonous Aquatic Probiotics

Isolation and Characterization of Autochthonous Microbes

In juvenile fish and shellfish, the autochthonous microbes may be isolated from the digestive tract after dissection, while distinguishing stomach and intestine regions. The microbes adherent to epithelial cells can be separated from those adherent to mucus, and from those transient in the lumen (Westerdahl *et al.*, 1991). These methods are not applicable to larvae and live food organisms, but the external surface of larval fish may be washed with a 0.1% benzalkonium chloride saline solution to differentiate the microbes adherent to the external surface from those present in the gut (Blanch *et al.*, 1997). Many microbes may be isolated on selective media (Pratt and Reynolds, 1973; Flint, 1985; Jeppesen, 1995; Donovan & Van Netten, 1995). Then the isolates are characterized by proper methods (Roth *et al.*, 1962; Hansen and Sorheim, 1991; Holt *et al.*, 1994; Bertone *et al.*, 1996; Austin *et al.*, 1997; Tannock, 1999).

Pioneering studies

The first successful report seems to be attributed to Maeda and Liao (1992), who isolated a strain “PM-4” from the rearing water of larval *Pen. monodon*, with good survival and molting rate. The bacterium, identified as *Thalassobacter utilis* (Nogami *et al.*, 1997.), was used for the bio control of the larval rearing of *Pen. monodon* (Maeda and Liao, 1992; Maeda *et al.*, 1997) and the swimming crab, *Portunus trituberculatus* (Nogami and Maeda, 1992; Nogami *et al.*, 1997).

Griffith (1995) reported that shrimp larvae reared in Ecuadorian hatcheries were affected by a disease characterized by a change in the bacterial population. The proportion of *Vibrio alginolyticus* decreased, whereas *Vibrio parahaemolyticus* increased. The strain of *V. alginolyticus* was isolated and used as probiotic in

many hatcheries, where shrimp survival was restored to the level obtained before disease outbreak. Austin *et al.*, (1995) investigated the probiotic effect of this strain, and these authors reported that cells of *Vibrio ordalii* lost their viability within 3 h after the introduction of freeze-dried supernatant of probiotic culture into the suspension medium. *V. anguillarum* and *Aeromonas salmonicida* were also inhibited, but to a lesser extent.

The probiont survived in the intestine of Atlantic salmon for at least 3 weeks, and a preliminary bath with this probiont improved the survival of salmon challenged with pathogens. This provides an example of what might be expected from probiotics: (1) antagonism to pathogens, (2) gut colonization, with possible adhesion to intestinal mucus, and (3) increased resistance of the host to pathogens.

Advantages of The use of Probiotics and Mode of Action

Production of Inhibitory Compounds

Probiotic bacteria release a variety of chemical compounds that are inhibitory to both gram-positive and gram negative bacteria/these include bacteriocins, siderophores, lysozymes, proteases, hydrogen peroxides etc. Lactic Acid Bacteria (LAB) are known to produce compounds such as bacteriocins that are inhibitory to other microbes (Saurabh *et al.*, 2005).

Probiotic bacteria produce substances with bactericidal or bacteriostatic effects on other microbial populations such as bacteriocins, hydrogen peroxide, siderophores, lysozymes, proteases, among many others (Servin, 2004; Panigrahi & Azad, 2007; Tinh *et al.*, 2007). In addition, some bacteria produce organic acid and volatile fatty acids (e.g. lactic, acetic, butyric and propionic acids), that can result into the reduction of pH in the gastrointestinal lumen, thus preventing growth of opportunistic pathogenic microorganisms (Tinh *et al.*, 2007).

Antibacterial Activity

Several probiotics in aquaculture have been documented possessing antibacterial activity against known pathogens. For example, probiotic *L. lactis* RQ516 that is being used in tilapia (*Oreochromis niloticus*) exhibited inhibitory activity against *Aeromonas hydrophila* (Zhou *et al.*, 2010). It was also shown by Balcazar *et al.*, (2008)

that probiotic *L. lactis* had antibacterial activity towards two fish pathogens namely, *Aeromonas salmonicida* and *Yersinia ruckeri*.

The potential of probiotic including *Lactobacillus plantarum* (LP1, LP2), *Saccharomyces cerevisiae* (SC3), *Candida glabrata* (CG2), *L. lactis* subsp. *lactis* (LL2) and *Staphylococcus arlettae* (SA) isolated from an indigenous fish sauce in Malaysia showed high inhibitory activity on *Staphylococcus aureus* and *Listeria monocytogenes* (Dhanasekaran *et al.*, 2008).

Antiviral activity

The knowledge on antiviral activity of probiotics has been raised in recent years (Lakshmi *et al.*, 2013). For example, *Pseudomonas*, *Vibrio*, *Aeromonas* spp. and *Coryneforms* had antiviral activity against Infectious Hematopoietic Necrosis Virus (IHNV) (Kamei *et al.*, 1988 & Li *et al.*, 2009) demonstrated that feeding with a *Bacillus megaterium* strain increased the resistance to White Spot Syndrome Virus (WSSV) in the shrimp *Litopenaeus vannamei*. It was documented that probiotics such as *Bacillus* and *Vibrio* sp. positively protect shrimp *L. vannamei* against WSSV (Balcazar, 2003). Application of *Lactobacillus* probiotics as a single strain or mixed with Sporolac improved disease resistance against lymphocystis viral disease in olive flounder (*Paralichthys olivaceus*) Harikrishnan *et al.*, (2010).

Antifungal Activity

There are few studies regarding the antifungal effect of probiotics. Lategan *et al.*, (2004) isolated *Aeromonas* media (strain A199) from eel (*Anguilla australis*) culture water and were observed to have a strong inhibitory activity against *Saprolegnia* sp. In a separate study, *Pseudomonas* sp. M162, *Pseudomonas* sp. M174 and *Janthinobacterium* sp. M169 enhanced immunity against *Saprolegniasis* in rainbow trout. Atira *et al.*, (2012) demonstrated that *L. plantarum* FNCC 226 exhibited inhibitory activity against *Saprolegnia parasitica* A3 in catfish (*Pangasius hypophthalmus*).

Competition for Adhesion Sites

Probiotic organisms compete with the pathogens for the adhesion sites and food in the gut epithelial surface and finally prevent their colonization (Vanbelle *et al.*, 1990). Adhesion capacity and growth on or in intestinal or

external mucous has been demonstrated *in vitro* for fish pathogens like *Vibrio anguillarum* and *Aeromonas hydrophila* (Krovacek *et al.*, 1987).

Competition for Nutrients

Probiotics utilizes nutrients otherwise consumed by pathogenic microbes. Competition for nutrients can play an important role in the composition of the microbiota of the intestinal tract or ambient environment of the cultured aquatic organisms (Ringo & Gatesoupe, 1998). Hence, successful application of the principle of competition to natural situation is not easy and this remains as a major task for microbial ecologists.

Source of Nutrients and Enzymatic Contribution to Digestion

Some researchers have suggested that probiotic microorganisms have a beneficial effect in the digestive processes of aquatic animals. In fish, it has been reported that *Bacteroides* and *Clostridium* sp. have contributed to the host's nutrition, especially by supplying fatty acids and vitamins (Sakata 1990). Some microorganisms such as *Agrobacterium* sp., *Pseudomonas* sp., *Brevibacterium* sp., *Microbacterium* sp., and *Staphylococcus* sp. may contribute to nutritional processes in Arctic charr (*Salvelinus alpinus* L.) (Rango *et al.*, 1995). In addition, some bacteria may participate in the digestion processes of bivalves by producing extracellular enzymes, such as proteases, lipases, as well as providing necessary growth factors (Prieur *et al.*, 1990). Similar observations have been reported for the microbial flora of adult penaeid shrimp (*Penaeus chinensis*), where a complement of enzymes exists for digestion and synthesis compounds that are assimilated by the animal (Wang *et al.*, 2000). Microbiota may serve as a supplementary source of food and microbial activity in the digestive tract may be a source of vitamins or essential amino acids (Dall & Moriarty, 1983).

Enhancement of Immune Response

The ability of the administered probiotic to modulate the nonspecific immune responses thus, increase disease resistance during bacterial infections in aquatic animals was documented by several studies (Balcazar *et al.*, 2006; Gatesoupe, 2008). Recent studies have focused on the possible role of probiotics in immune system functions. Gatesoupe (2008) reported that feed supplemented by selected bacterial probiotics caused an

increase in some cellular and humoral parameters. Villamil *et al.*, (2002) found that *Lactococcus lactis* caused the higher increases in immune functions of turbot (*S. maximus*). Later, Villamil *et al.*, (2003) proved that the whole cell, fractions whole cell and the extra cellular products of LAB such as nosing act as Immunomodulator in turbot (*Scophthalmus maximus*), the increase was in chemiluminescence's and nitric oxide production in a dose and time dependant manner.

In shrimp, Balcazar *et al.*, (2003) increased the resistance of shrimp, *Litopenaeus vannamei*, against *Vibrio harveyi* and white spot syndrome by administration of a mixture of *Bacillus* and *Vibrio* spp. Chiu *et al.*, (2007) reported increases in activities of superoxide dismutase (SOD), phenoloxidase (PO), respiratory burst as well as the clearance efficiency of *Vibrio alginolyticus*, in addition, a recorded increase in the mRNA transcription of prophenoloxidase (proPO), and peroxinectin (PE) as immune profile factors in white shrimp, *Litopenaeus vannamei*, when treated with *Lactobacillus plantarum* supplemented food. Liu *et al.*, (2012) proved that *B subtilis* was able to survive in grouper, *Epinephelus coioides*, and posterior intestines during the feeding period; the relative survival percentages of fish challenged with *Streptococcus* spp. and iridovirus were increased in time and dose dependent manner. Significant increases in respiratory bursts, phagocytic activity, superoxide dismutase (SOD) level of leukocytes and serum alternative complement activity (ACH 50) when compared with controls.

Activating the immune system is costly operation (Martin *et al.*, 2002). In teleosts, probiotics can positively stimulate various immune hematological parameters such as mononuclear phagocytic cells (monocytes, macrophages) and polymorph nuclear leukocytes (neutrophils) and NK cells (Balcazae, 2003). Probiotics actively stimulate the proliferation of B lymphocytes, thus elevation of immunoglobulin level in both *in vitro* and *in vivo* conditions, Elevation of immunoglobulin level by probiotics supplementation is reported in many animals and fish (Pirarat *et al.*, 2011; Nayak *et al.*, 2007; Panigrahi *et al.*, 2004).

Probiotics can effectively stimulate phagocytosis through alarming of the pahgocytic cells, the latter is accountable for early intervention through activation of inflammatory responses before antibody production and plays a crucial role in antibacterial defenses in numerous fish and shellfish species (Roman *et al.*, 2012; Touraki *et al.*,

2012). Respiratory burst activity is an important innate defense mechanism of fish. The findings of respiratory burst activity following probiotics treatment in fish are typically contradictory. Whereas some studies indicate probiotics do not have important impact on this non-specific defense reaction of fish (Nayak *et al.*, 2007; Mohapatra *et al.*, 2012; Diaz *et al.*, 2009). Many in vitro and in vivo studies showed important increase in Respiratory burst activity by numerous probiotics in several aquatic animals as well as fish (Sharifuzzaman & Austin 2009; Ibrahim *et al.*, 2012). Lysozyme is one of the important bactericidal enzymes of innate immunity is an indispensable tool of fish to fight against infectious agents (Lindsay, 1986). Lysozymes can be found in serum, mucosal membranes of skin and intestine. Probiotics either single or in combination are found to trigger the lysozyme level in teleosts. The enhancement of lysozyme level was recorded by various types of probiotics (Vine *et al.*, 2006; Gatesoup, 2008; Panigrachi *et al.*, 2004; Kim & Austin, 2006; Song *et al.*, 2006).

The non-specific immune system can be stimulated by probiotics. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria to rainbow trout enhanced the resistance of fish to *vibriosis*, by increasing the phagocytic activity of leucocytes (Sakai *et al.*, 1995; Rengpipat *et al.*, 2000) reported that the use of *Bacillus sp.* (strain S11) has provided disease protection by activating both cellular and humoral immune defenses in tiger shrimp (*Penaeus monodon*). Balcazar (2003) demonstrated that the administration of a mixture of bacterial strains (*Bacillus* and *Vibrio sp.*) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harveyi* and white spot syndrome virus. This protection was due to a stimulation of the immune system, by increasing phagocytosis and antibacterial activity. In addition, Nikoskelainen *et al.*, (2003) showed that administration of a lactic acid bacterium *Lactobacillus rhamnosus* (strain ATCC 53103) at a level of 105 cfu g⁻¹ feed, stimulated the respiratory burst in rainbow trout (*Oncorhynchus mykiss*).

Improves Water Quality

There are no serious problems for water quality during the initial stages of farming aquatic organisms, when the stocked organisms are small and their metabolism rate and amounts of supplementary feed are low. However, with the progress of culture the organisms grow, leading

to a rapid increase in biomass, and water quality deteriorates mainly as a result of the accumulation of metabolic waste of cultured organisms, decomposition of unutilized feed, and decay of biotic materials (Prabhu *et al.*, 1999). At this time, the application of a group of beneficial microorganisms (such as *Lactobacillus*, *Bacillus*, *Nitrosomonas*, *Cellulomonas*, *Nitrobacter*, *Pseudomonas*, *Rhodoseudomonas*, *Nitrosomonas* and *Acinetobacter*) would be very useful for controlling the pathogenic microorganisms and water quality (Prabhu *et al.*, 1999; Shariff *et al.*, 2001; Irianto & Austin 2002).

By definition, bacteria added directly to pond water are not probiotics, and should not be compared with living microorganisms added to feed (Rengpipat *et al.*, 2003). Many workers have evaluated some specific microorganisms as biological improvers for water quality: Douilett (1998) used a probiotic additive consisting of a blend of bacteria in liquid suspension in intensive production systems.

The probiotic blend improved water quality in fish and crustacean cultures by reducing the concentration of organic materials (OM) and ammonia. This procedure was accomplished by a series of enzymatic processes carried out in succession by the various strains present in the probiotic blend. The addition of this blend to culture systems reduced the concentration of *Vibrio* strains and thus controlled diseases caused by *Vibrio* strains. In addition, *Bacillus* spp have been evaluated as probiotics, with uses including the improvement of water quality by influencing the composition of water-borne microbial populations and reducing the number of pathogens in the vicinity of the farm species. Thus, the Bacilli are thought to antagonize potential pathogens in the aquatic environment (Irianto & Austin, 2003).

Bacterial species belonging to the genera *Bacillus*, *Pseudomonas*, *Nitrosomonas*, *Nitrobacter*, *Acinetobacter* and *Cellulomonas* are known to help in the mineralization of organic water and in reducing the accumulation of organic loads (Shariff *et al.*, 2001).

Furthermore, there are many reports of the use of microbial products in aquaculture ponds for increasing the removal rate of ammonia. Prabhu *et al.*, (1999) used some microorganisms in a shrimp farm to evaluate them as a factor for controlling the water quality. According to the results of this study, all factors of water quality parameters were at optimum levels in the experimental ponds compared with the control.

Enhance Human Consumption

The use of live microorganisms to enhance human health is not new. For thousands of years, long before the discovery of antibiotics, people have been consuming live microbial food supplements such as fermented milks. According to Ayurveda, one of the oldest medical sciences that date back to around 2500 BC, the consumption of yoghurt is recommended for the maintenance of overall good health. A scientific explanation of the beneficial effects of lactic acid bacteria present in fermented milk was first provided in 1907 by the Nobel Prize-winning Russian physiologist Eli Metchnikoff.

In his fascinating treatise 'The Prolongation of Life', Metchnikoff states that, 'The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes' (Talwalkar, 2003). He proposed that the acid-producing organisms in fermented dairy products could prevent 'fouling' in the large intestine and thus lead to a prolongation of the life span of the consumer (Heller, 2001). Probiotics have a great variety of effects on human health. Probiotic therapy could be used for applications such as: modulation of the intestinal microbial communities, immune modulation, controlling allergic diseases, treating diseases related to the gastrointestinal tract such as inflammatory bowel disease, and controlling colorectal cancer and constipation (Ouweland *et al.*, 2002).

Probiotics in Aquaculture Management

These organisms can be administered to the aquaculture management through feeding, injection or immersion of the probiotic bacteria (Irianto & Austin, 2002).

Application in Feed

Probiotics are applied with the feed and a binder (egg or cod live oil) and most commercial preparation contain either *Lactobacillus sp* or *Saccharomyces cerevisiae* (Abidi, 2003). According to FAO and WHO guidelines, probiotic organisms used in food must be capable of surviving passages through the gut i.e. they must have the ability to resist gastric juices and exposure to bile (Senok *et al.*, 2005). Furthermore they must be able to proliferate and colonize the digestive tract and they must

be safe, effective and maintain their effectiveness and potency for the duration of the shelf life of the product (Senok *et al.*, 2005).

Direct Application to Pond Water

The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. lecheniformis*, *Nitrobacter sp*, *Aerobacter* and *Sacharomyces cerevisiae*. Application of probiotic through water of tanks and ponds may also have an effect on fish health by improving several qualities of water, since they modify the bacteria composition of the water and sediments (Ashraf, 2000; Venkateswara, 2007).

Application through Injection

Application of probiotics by injection is a possibility. Austin *et al.*, (1995) suggested the possibility of freeze-drying the probiont like vaccine and applied either through bathing, or injection. Yassir *et al.*, (2002) has demonstrated the experimental administration of probiotic *Micrococcus luteus* to *Oreochromis niloticus* by injection through intra peritoneal route which had only 25% mortality as against 90% with *Pseudomonas* using the same route. According to (Yassir *et al.*, 2002; Nikoskelainen *et al.*, 2003) the use of probiotics stimulate *Rainbow trout* immunity by stimulating phagocytes activity, complement mediated bacterial killing and immunoglobulin production (Noh *et al.*, 1994).

Perspectives of Development

The advantage of probiotics over antibiotics was discussed by Moriarty (1998), but most attention has been hitherto directed towards the production of inhibitory substances by the probiotics. The risk to select probiotic-resistant pathogens must not be underestimated, and it is particularly important to search for diversified antagonistic properties, which may lower the risk of multi-resistance. For example, the ability of some probiotics to adhere to intestinal mucus may block the intestinal infection route common to many pathogens (Evelyn, 1996).

Antagonism may be also due to competition for nutrients that favour the growth of probiotics, or the expression of their inhibitory effects. Competitive exclusion has been mentioned as a possible mechanism for probiotic effects,

in reference to “highly selective substrate-limited growth conditions” (Dolfing and Gottschal, 1997). Iron is required by most organisms, and its availability in animal tissues may be virulence factor for pathogens. Smith and Davey (1993) suggested that the growth inhibition of *Ae. Salmonicida* by *Ps. fluorescens* was due to competition for free iron.

The antibacterial activity of *Bacillus sp* isolated by Sugita *et al.*, (1998) was also attributed at least partly to a siderophore. It may be therefore important to favour the expression of such siderophore-mediated probiotic effects by adjusting the dietary supply to meet but not exceed the requirement of the host. Iron is often supplemented in excess in fish diets, and for example, iron limitation changed the microbiota without detrimental effect on Seabass larvae (Gatesoupe *et al.*, 1997).

Other nutrients may affect the intestinal microbiota, though they are essential to aquatic animals but not to microbes. For example, the dietary polyunsaturated fatty acids seemed to influence the proportion of lactic acid bacteria in the gastrointestinal tract of Arctic charr (Ringø, 1993; Ringø *et al.*, 1998).

Suggestions for Further Directions

In the last decades, fish performance has improved considerably by the prophylactic use of probiotics as a biological control agents. The optimal conditions for probiotics to survive, colonize, proliferate and provide their effects to the hosts properly in a particular environment needs to be considered, because the term ‘one size fits all’ cannot be applied to probiotics. There needs to be specific probiotic strains/species for target fish species in particular environments. Therefore, further work is needed to produce more detail to increase knowledge on particular probiotics for specific fish species.

As both Gram-positive and Gram-negative bacteria can be used as probiotics, it is of concern in the horizontal gene exchange to other animals including humans (Newaj- Fyzul *et al.*, 2014). Resistance plasmids encoding for antibiotic resistance genes were transferred between pathogen and non-pathogenic Gram negative bacteria in sea water (Salyers, 1995; Moriarty, 1999). A consideration of the use of probiotics as antibiotics is needed as in many cases they are ineffective owing to an increase in virulence of pathogens.

The issue of promoting the transfer of antibiotic resistance to human pathogens because of the use of probiotics needs further studies to provide evidence (Salyers, 1995) and prevent this. An in-depth research on probiotics should focus on other molecular methods to better understand the modes of action. Quorum sensing, different staining methods, transmission electron microscope, scanning electron microscope, polymerase chain reaction, fluorescent in situ hybridisation (FISH), gnotobiotic animals and high through genomes technology could be used to create a better explanation of the present doubts in (i) adherence and colonization of probiotic and pathogenic bacteria, (ii) interactions between them within the digestive tracts, (iii) interaction between probiotics and host mucosa, (iv) gene expression and mucosal tolerance, (v) microvilli density and length, (vi) gene exchange or transfer. In manipulation of bacterial populations, the question is whether or not the domination of probiotics over other microbial populations by application of probiotics is correct, as they share the same living conditions.

Quorum sensing is used to investigate the inhibition property of probiotics to other bacterial communities. To investigate the domination of potential probiotic ability, the FISH technique is used as a potential tool to characterize their dynamics and efficiency in the control of pathogenic bacteria (Del’duca *et al.*, 2013 & Lamari *et al.*, 2013) proposed that the evaluation of probiotics should take into account ontogenetic chronology for improving larval quality. Some studies have proved that the use of selected probiotics can be an alternative method for the protection of aquatic animals against diseases. However, farmers cannot predict when the onset of disease may occur to provide probiotic feeding in the weeks prior to infection. Therefore, further work on the effects of treatment is required if the onset has already occurred (Merrifield *et al.*, 2010b). It is noted that a screen of promising probiotics plays a significant role in the selection of appropriate probiotics in aquaculture, as positive results in vitro sometimes fail to determine at in vivo effects (Kesarodi-Watson *et al.*, 2008). Moreover, the longevity of the health effect of probiotics is often uncertain. The fate of live probiotics in the aquatic environment is uncertain (Newaj-Fyzul *et al.*, 2014).

Although there are no data to support short-term-cyclic probiotic feeding strategies, it is assumed that this technique may avoid overstimulating the immune response whilst maintaining a level of protection or

immune stimulation. Therefore, further research should investigate this application strategy properly (Merrifield *et al.*, 2010b). Although synbioticum (Liu *et al.*, 2010), and synbiotics (Rodriguez-Estrada *et al.*, 2009) bring benefits to the hosts, they also need further investigation on kinds, proportions, time, and mixture methods.

Probiotic bacteria can improve the utilization of feed with a lower FCR by producing digestive enzymes, while the aquaculture sector is facing the problem of a shortage of fish meal for protein sources.

Therefore, the role of probiotics in aquaculture becomes vital in collaboration with an alternative method to animal protein, by substituting plant protein sources. It is essential to investigate the metabolic capabilities of probiotics in the degradation of anti-nutrients to improve the nutritional value (Merrifield *et al.*, 2010b), particularly in plant protein sources.

Dosage dependent studies are currently limited and somewhat contradictory. Further investigations are also needed before giving guidelines with any degree of confidence (Merrifield *et al.*, 2010b). In addition, overdosages or prolonged administrations of probiotics induce immunosuppression of continuous responses of the hosts (Sakai, 1999). Although there are not many evidences about prolonged administration of probiotics in aquaculture, the Sakai (1999)'s hypothesis that on converse results or even death, if probiotics are applied at over dosages, over a long period of time, and indiscriminate frequency, need further studies. These investigations can also help to maintain an efficient immune system, which is reflected in fish quality and productivity.

The application of probiotics in aquaculture shows promise, but needs considerable efforts of research. The first question, unanswered in many cases, is the fate of the probiotic in rearing medium and in gastrointestinal tract. Immunological and molecular probes will be useful tools to trace the probiotic cells (Ringø *et al.*, 1996; Austin, 1998; O'Sullivan, 1999). It is essential to investigate the best way of introduction and the optimal dose, and technical solutions are required, especially to keep the probiotic alive in dry pellets.

The spores of *Bacillus spp.* are especially easy to introduce in dry food, and this is an additional advantage of these promising candidate probiotics (Moriarty, 1998; Queiroz and Boyd, 1998; Kennedy *et al.*, 1998;

Rengpipat *et al.*, 1998; Sugita *et al.*, 1998). Lactic acid bacteria are also good candidates, and further studies are necessary to evaluate the interest of yeasts as probiotics.

The bacteria normally dominant in healthy animals may be sources of probiotics, but there are many potential pathogens among *Vibrionaceae* and pseudomonads. It may be wise to carry out long-term surveys to make sure that the bacteria keep innocuous, without risk of apparition of potentially detrimental mutants.

The influence of probiotics on gastrointestinal microbiota remains poorly described, but further investigation may be expected with the propagation of molecular approaches to analyse bacterial communities (Raskin *et al.*, 1997; Wallner *et al.*, 1997; Hugenholtz *et al.*, 1998).

Recommendations

Fish farmers and other stakeholders in aquaculture management should make use of probiotics because of its colonization ability as preventive measures against over dependency on antibiotic therapy which is costly. Fish farmers are also encouraged to incorporate probiotics in their feed formulations because of its importance in digestibility improvement. Close network of aquaculture experts, fish nutritionists and microbiologists necessary to develop such aquatic foods.

Author Contributions

K. P. Renuka: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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