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A review: Application of probiotics in aquaculture

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***Abstract*— *Probiotics can be used as beneficial alternative to enhance the aquaculture production in sustainable way. Selection of the right strain and dose for particular aquaculture species is necessary for the desirable benefits of probiotics application. Probiotics can be administrated as water additives, feed additives and through injection of which as feed additives is commonly used in aquaculture. Application of probiotics has various benefits in aquaculture production as improve the growth performance, enhances the feed utilization, enhance the immune defense against pathogens, disease resistance, improve water quality and enhance stress tolerance capacity. Thus, application of probiotics in aquaculture can be used at the farm level to enhance the economic performance of the aquaculture species.*

Keywords*— *aquaculture, probiotics, non- pathogenic, non-toxic.

I. INTRODUCTION

Aquaculture is the farming of aquatic organisms in both coastal and inland areas involving interventions in the rearing process to enhance production (FAO,2019). The global aquaculture production of fish has grown tremendously during the last seventy years from a production less than a million ton in the early 1950s to 82 million tons with the value of USD 250 billion in 2018 (SOFIA, 2020). The contribution of aquaculture to world fish production reached 46 percent in 2018, up from 25.7 percent in 2000 (SOFIA, 2020). Global food fish consumption increased at an average annual rate of 3.1 percent from 1961 to 2017, a rate almost twice that of annual world population growth that is 1.6 percent for the same period, and higher than that of all other animal protein foods(meat, dairy, milk etc.), which increased by 2.1 percent every year (SOFIA, 2020). In 2017, fish consumption accounted for 17 percent of the global population's intake of animal proteins and 7 percent of all proteins consumed (SOFIA, 2020). The above data shows that the world aquaculture grow at the faster rate with an average annual growth rate of 5.3 percent per year in the period 2001-2018 (SOFIA, 2020).From the above data, we can conclude that the global fish consumption increased at the higher rate than other animal protein source. Hence, to meet the growing demand of increasing global population, aquaculture production practices have been intensified to a greater extent both in technological and practical measures (Taun et al., 2013).

With the increase in the intensification and commercialization of aquaculture production to meet the demand, aquaculture faces many challenges such as combating diseases and epizootics, brood stock improvement and domestication, development of appropriate feedstuffs and feeding mechanisms, hatchery and growout technology as well as water quality management with the increase in the intensification and commercialization of aquaculture production (Subasinghe, 2003). Out of these, disease outbreaks are now primary constraints to the culture of many species that affect the aquaculture production, supressing both economic and social development in many countries (Qi et al. 2009, Taun et al. 2013). Diseases caused serious economic losses to the finfish aquaculture in the world (Mustafa et al. 2001, Johnson et al. 2004, Sahoo et al. 2013, Monir et al. 2015, Taveres-Dias et al. 2017), globally estimated in US$ 1.05 to US$ 9.58 billion/year (Shinn et al. 2015).Over the years, antibiotics in aquaculture have been used as traditional strategy for the control and prevention of fish diseases and also for the improvement of growth and efficiency of feed conversion. The use of antimicrobials in aquaculture basically started with the work of Gut sells (1946) who recognized the prospective use of antibiotics i.e. sulphanamides for combating furunculosis. The joint

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FAO/OIE/WHO expert meeting on antimicrobial use and antimicrobial resistance in aquaculture, 2017 conclude the two main hazards of antimicrobial used in aquaculture i.e. antimicrobial residues and antimicrobial resistance. The indiscriminate use of antibiotics results in the prevalence of antibiotic resistance microorganisms and imbalance in the gut microflora, which affects fish health and residual deposition in the fish muscle: a potential health risk to consumer (Williams, 2017). Due to the threat related to the use of antibiotics in aquaculture, an alternative approach to manage fish health in the aquaculture industry is probiotics, a microbial intervention approach, which has been found to improve not only fish health but in many instances fish growth (Williams, 2017). Many aqua culturists have found the different non- antibiotic agents, out of them probiotics play the significant role for the health maintenance in the intensified aquaculture. Probiotics can be used to enhance growth, improve feed utilization, enhance disease resistance and immune response and improve the water quality in aquaculture. This review summarizes the selection of probiotics, types of probiotics, administrative methods and benefits of probiotics application in aquaculture species.

II. HISTORY AND DEFINITION OF PROBIOTICS

The term probiotics was first used by Lilley and Stillwell in 1965 to describe substances secreted by one microorganism which stimulated the growth of another (Fuller,1992) which thus meant the exact opposite of ‘antibiotics’. Later on, it was subsequently used by Sperti in 1971 to describe ‘tissue extracts which stimulated microbial growth’ (Fuller, 1992). The term probiotics is derived from Greek word ‘Biotikos’ meaning ‘for life’ which was first coined by Parker (1974). He defined probiotics as ‘organisms and substances which contribute to intestinal microflora’ (Fuller et al., 1992). In an attempt to improve the definition, Fuller (1989) revised the definition of probiotics as ‘A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance’ (Fuller, 1992).After that, definition proposed by Havenaar & Huis in 1992 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, Guarner and Schaafsma assumed that probiotics are live microorganisms which, when consumed in adequate amounts, confer health benefits to the host (Cruz et al., 2012). According to Moriarty 1998, the definition of probiotics applied to aquaculture includes bacteria which improve water quality on addition to water and/or inhibit pathogens in the water (Rengpipat, 2005). Gatesoupe in 1999, defined them as “microbial cells administered in a certain way, which reaches the gastrointestinal tract and remain alive with the aim of improving health” (Gatesoupe, 1999, Cruz et al., 2012). The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) defined probiotics as living microorganisms, which once administered in appropriate amounts, confer a health profit on the host (FAO/WHO, 2001). Kosaza in 1986 made the first empirical application of probiotics in aquaculture , considering the benefits exerted by the use of probiotics on human and poultry (cruz et al., 2012). Recently, Lazado and Caipang in 2014, proposed that probiotics under an aquaculture understanding be defined as ‘live or dead, or even a component of the microorganisms that act under different modes of action in conferring beneficial effects to the host or to its environment (Lazado and Caipang, 2014).

III. SELECTION OF PROBIOTICS

The proper selection of probiotics is very critical because in appropriate strains can lead to undesirable effects in the host (Abudurasak Ige, 2013). The primary objective of selecting probiotics is that they should be safe and produced desirable benefits. Additionally, they must retain their ability during production, manufacturing, distribution and storage prior reaching to the consumer (Shewale et al., 2014). A good and successful probiotics should have a few specific characteristics that are listed below.

Characteristics of good probiotics (Fuller, 1989; Michael, 2014)

i) It should be a strain capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease.

ii) It should be non- pathogenic and non-toxic.

iii) It should be present as viable cells, preferably in large number.

iv) It should be capable of surviving and metabolizing in the gut environment, e.g. resistance to low pH and organic acids.

v) It should be stable and capable of remaining viable for longer periods under storage and field conditions.

The United Nations has recommended a number of specifications that should be considered when a probiotic product is selected and approved which are listed below (Aly, 2009):

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i)Viability of the probiotic to survive when passage through the Gastro intestinal tract (GIT) should be demonstrated.

ii) Colonization by the probiotic should occur when present in the GIT of the host

iii) Competition of the probiotic culture against pathogenic bacteria for the attachment on intestinal surface

iv) The probiotic should be efficient in inhibiting pathogenic bacteria according to in vitro tests

v) Probiotics should show resistance against other sanitary agents or disinfectants

vi) Idesntification of probiotics products should be indicated on the label by genus and species name according to international nomenclature.

vii) Doses and expiration date should be indicated on the label and

viii) Data indicating that the product will not infect immune compromised animal is desirable.

IV. TYPES OF PROBIOTICS

The different types of probiotics are tabulated below in the table 1:

Table 1 (Rao, 2010)

Types	Description
Non- viable probiotics	These are dead.
Freeze- dried probiotics	These will die rapidly upon leaving refrigeration.
Fermentation probiotics	These are produced through fermentation.
Viable probiotics	This is live with guaranteed number of organisms, have a protocol for counting and to be very stable and efficacious.

V. ADMINISTRATIVE METHODS

Probiotics in aquaculture can be administered through different routes as feeding, injection or direct immersion in water (Michael, 2014) which can be applied singly or in combination (Hai et al., 2015).

5.1) Feed additives, water additives and injection

The most commonly used methods for administrating probiotic mixtures is incorporation into the feed (92.8%), followed by direct incorporation into the water (4.8%) and in live food (1.6%) (Melo et al., 2020). Some bacterial, fungal strains can be mixed with feeding pellets or by encapsulating into live feed stock or administered orally to feed for rearing animals (Das, Mandal and Haque, 2017). Probiotics including bacterial strains, yeast and extracted substances are generally supplied as feed additives in aquaculture (Taun et al., 2013). Probiotic *Lactibacillus plantarum* CR1T5 applied as feed additives improved the growth performance and non-specific immunity in black eared catfish (Silarudee et al., 2019). Feed additives such as probiotic, *Bifidobacterium* strains enhanced the growth performance and nutrient utilization of rainbow trout fry (Sahandi et al., 2018). Furthermore, probiotics can also be administrated as water additives (Gobi et al., 2016, Gupta et al., 2016). Probiotic *Vibrio lentus* as water additives at the dose of 10^6 CFU/ ml in the sea bass significantly modified gene expression i.e. immune response, cell proliferation and death cell adhesion, ROS metabolism and iron transport (Schaek et al., 2017). In addition to above methods, application through injection can also be done. Injection of the probiotic *Enterobacter sp.* strain C6-6 through intramuscularl and intraperitoneal route enhanced the immunity in rainbow trout (Laptra et al., 2014).

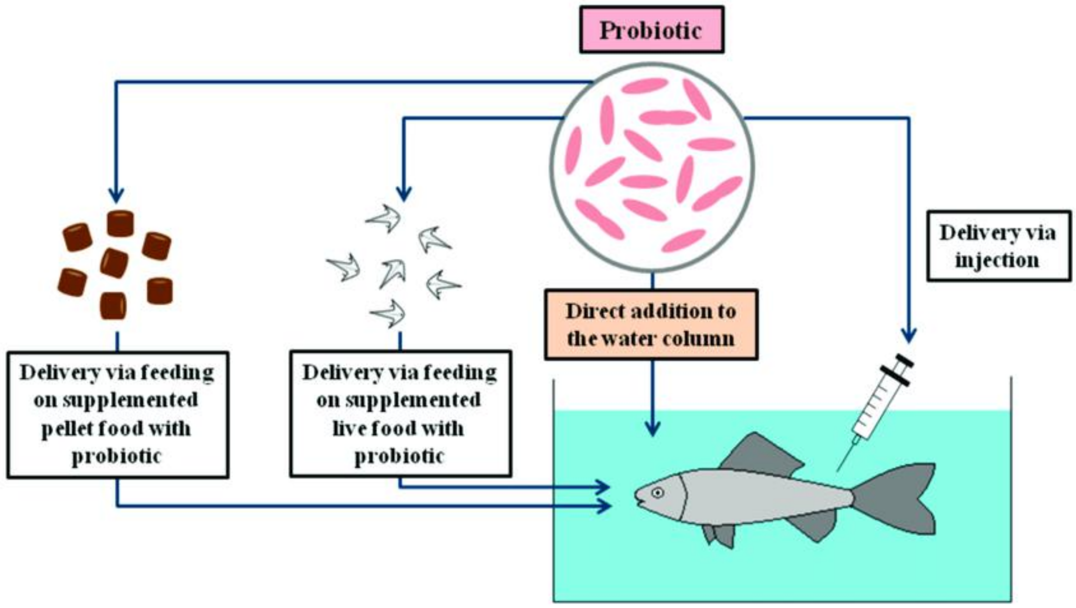

Fig.1: Different routes for administration of probiotic (Source: Jahangiri and Esteban, 2018)

5.2) Single and combination

Probiotics can be applied singly or in combination in different forms such as multistrain probiotics, probiotic with plant extract and probiotics with yeast extract (Shaibe Hossain, 2018). Most of the study on probiotics in aquaculture focused on use of single probiotics but the combination of probiotics is more beneficial. The advantage of multi-strain probiotics is that they are more sensitive towards pathogens and active against different aquaculture animals (Pannu et al., 2014). A positive effects of the multi-strain probiotics on the growth and survival of rohu was seen at hatchling and fry stages but not at later stages (Jha et al., 2014). The combination of *Bacillus megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211 adminstrated in feed showed best result than the single application of either probiotics in catfish *Clarias sp.* (Hamka et al., 2020). The combined use of Lactobacillus plantarum N11 and Bacillus velezensis H3.1 in Nile tilapia *(Oerochromis niloticus)* promoted higher survival rate (58.33%) than their single application i.e. *Lactobacillus plantarum* (54.17%) and *Bacillus velezensis* (41.67%) (Doan et al., 2018). The combination of probiotic *Bacillus coagulans* and plant extract *Mentha piperita* showed the higher growth performance, nutrient retention and immunity in *Catla catla* than their single application (Bhatnagar & Saluja, 2019).

5.3) Dosage: For the optimum effectiveness of the probiotic on the particular species, the appropriate dosage should be administrated. Appropriate probiotic levels depend on the probiont species, fish species and their physiological status, rearing conditions and the specific goal of the applications (Hai, 2015). Growth performance was improved when the rainbow trout is fed with probiotic *Bifidobacterium* strains @ 1×10^7 CFU/g but not with 3×10^7 CFU/g (Sahandi et al., 2018). This suggests that more probiotics in feed does not necessarily result in more increase in growth. Dose is an important consideration while supplementing functional nutrients in aquafeeds. Weight gain was reduced when more than 0.2% of dietary commercial probiotics is supplied to pabda catfish, *Ompok pabda* (Chowdhury et al., 2020). This study revealed that more than higher concentration might not be able to maintain overall body physiology of fish and it could create disturbances in carbohydrates and fat metabolism.

VI. BENEFITS OF PROBIOTICS IN AQUACULTURE

6.1) Improve feed utilization: Many research concluded that the use of probiotics results in the alteration of enzymes and hence improve feed utilization. Supplementatiom of *Lactobacillus pentosus* improved the feed utilization in white shrimp, *Litopenaeus vannamei* (Zheng and Wang, 2016). Incorporation of heat killed *Lactobacillus plantarum* at 50, 100 or 1000 mg/kg for 12 weeks significantly enhanced amylase, lipase and protease activity of Nile tilapia (Dawood et al., 2019). An elevation in protease, amylase, alkaline phosphatase and lipase activity was observed in narrow clawed crayfish, *Astacus leptodactylus* fed with *Lactobacillus plantarum* at the concentration of 10^7, 10^8 and 10^9 CFU/gm (Valipour et al., 2019).

6.2) Growth promoter : One of the most expected consequences of using bacterial probiotics is the direct effect of probiotic on the growth performance of fish either by direct increment in nutrition uptake or providing nutrients (Joel et al., 2020). The significant enhancement of growth performance by probiotics supplementation may be due to increase of releasing digestive enzymes and promote appetite, production of vitamins, breakdown of indigestible components as well as improvement of gut morphology (Doan et al., 2018). Probiotics has been widely used in shellfish culture as growth promter. Feed supplementation of *Bacillus subtilis* at 10^7 and 10^9 CFU/kg diet for 5 weeks efficiently improved the growth in pacific white shrimp, *Litopenaeus vannamei* (Kewacharoen & Srisapoome, 2019). Dietary supplementation of *Enterococcus faecalis* and *Pediococcus acidilacti* significantly improved weight gain and specific growth rate of mud crab, *Scylla paramamosain* (Yang et al., 2019). Similarly, probiotics is also used in ornamental fish. Dietary application of *Pediococcus acidilactici* promoted the growth performance in zebra fish (*Danio rerio*) without imparing its appetite (Ahmadifar et al., 2020).

Furthermore, probiotics is being widely used in finfish aquaculture as growth promoter. Supplementation of *Bacillus pumilus* on juvenile golden pompano, *Trachinotus ovatus* improved the weight gain and specific growth rate as growth parameter (Liu et al., 2019). Supplementation of *Bacillus circulans* PB7 in the formulated diets promoted the growth in *Catla catla* (Bandyopadhay and Mohapatra, 2009). Single or combined supplementation of *Bacillus megaterium* and *Pediococcus pentosaceus* improved the growth performance of catfish *Clarias sp.* (Hamka et al., 2020). Dietary supplementation of *Pediococcus pentosaceus* enhanced the growth rate in grass carp by increase in mucus secreting cells , elongated instestinal villi and influencing the gut microbiota and in common carp, *Cyprinus carpio* by enhancing the digestive enzymes activities (Gong et al., 2019, Ahmadifar et al., 2019). Dietary supplementation of *Lactobacillus plantarum* at the dose of 10^8 CFU/gm for 4 weeks enhanced the growth performance in Nile tilapia, *Oerochromis niloticus* (Zhai et al., 2017). The growth and feed utilization performance of *Ompok pabda* were significantly higher when dietary commercial probiotics was supplied due to the increment in the digestive enzyme activity, elevated health status and stimulation of gastric development (Chowdhury et al., 2020).

6.3) Increase disease resistance: Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of host, eg: Bacitracin and polymyxin produced by *Bacillus sp.* and hence enhance the disease resistance (Cruz et al., 2012, Rao, 2010). Supplementation of probiotic *Enterobacter sp*. Enhance the disease protection against *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Laptra et al., 2014). Two isolated intestinal autochthonous probiotic bacteria *Aeromonas veronii* and *Flavobacterium sasangense* enhanced disease resistance of Common carp against *Aeromonas hydrophila* (Chi et al., 2013). Supplementation of *Lactococcus garvieae* isolated from the raw cow milk at the dose of 10^7 cells/gm for 10 days increased resistance against *Staphylococcus aureus* in Nile tilapia (Abdelfatah & Mahbouh, 2018). Supplementation of *Lactococcus lactis* isolated from *Cromileptes ativelis* gut at the dose of 10^6, 10^8, 10^{10} CFU/g for 4 weeks enhanced resistance against *Vibrio harveyi* in *Cromileptes altivelis* (Sun et al., 2018). Banos et al., 2018 reported that administration of *Enterococcus faecalis* isolated from commercial probiotic @ 10^8 CFU/g for 30 days increased the disease resistance against *Lactococcus garvieae* in rainbow trout. Gong et al., 2019 reported that *Pediococcus pentosaceus* exhibit excellent antibacterial activity against several important fish pathogens including *Aeromonas hydrophila, Aeromonas veroni, Aeromonas sobria, Eelwardsiella tarda, Lactococcus garviae* and *Plesiomonas shigelloide*.

6.4) Enhancement of the immune response: Probiotics can enhance the various immunological parameters in aquaculture species. Probiotics can inhibit the pathogen infection by enhancing the host immune through the stimulation of body non-specific and cellular immunity (Hamka et al., 2020). Administration of viable lactic acid bacteria, *Lactococcus lactis, Leuconostoc mesenteroides* and *Lactobacillus sakei* enhanced both cellular and hormonal immune functions in rainbow trout by increasing the proportion of phagocitically active cells from head kidney and activating the complement receptor expression (Balcazar et al., 2007). Host associated probiotics *Lactobacillus plantarum* and *Bacillus velezensis* enhanced the innate immune pramameters as skin mucus lysozyme and peroxidase activity, serum lysozyme, serum peroxidase, alternative complement, phagocytosis and respiratory brust activities in nile tilapia (Doan at al., 2018). Supplementation of host gut derived probiotic, *Bacillus pumilus* on juvenile golden pompano, *Trachinotus ovatus* increased lysozyme activity and total protein of fish (Liu et al., 2019). Dietary supplementation of

Lactobacillus plantarum at 10^8 and 10^9 CFU/g significantly improved complement activity after 15 days and significantly enhanced respiratory brust activity and lysozyme activity in black eared catfish, *Pangasius larnaudii* after 30 days and 45 days of feeding respectively (Silarudee et al., 2019). Feed supplementation of *Bacillus subtilis* at 10^7 and 10^9 CFU/kg diet in white shrimp, *Litopenaeus vannamei* enhanced immune responses through phagocytic activity and clearance efficiency (Kewacharoen & Srisapoome, 2019). Ahmadifar et al., 2019 reported that dietary supplementation of *Pediococcus pentosaceus* in common carp result in increased in red blood cells, white blood cells and hematocrit as well as total serum antibody level, alternative complement, protease and lysozyme activities and antibacterial activity. *Saccharomyces cerevisiae* supplemented in date palm seed meal enhanced the lysozyme and phagocytic activity in Nile tilapia (Dawood et al., 2020).

6.5) Improve water quality: Probiotic bacteria can directly uptake or decompose the organic matter or toxic material in the water improving the quality of the water. Probiotics can decompose the excreta of fish or prawns, remaining food materials, remains of the plankton and other organic materials to CO_2, nitrate and phosphate and hence improve the nutrient cycle that maintain a good water quality environment for cultured animals (Rao, 2010). Nimrat et al., 2012 reported that administration of mixed Bacillus probiotics significantly enhanced the water quality for the levels of pH, ammonia and nitrite of culture water during rearing of white shrimp (Nimrat et al., 2012). The water addition of probiotic *Bacillus subtilis* @ 10^3-10^5 CFU/ml effectively reduced the total ammonia and enhanced water quality in shrimp culture (Kewacharoen & Srisapoome, 2019). Administration of probiotics *Bacillus cereus* and *Pediococcus acidilactici* @10^6 CFU/ml to the pond water positively decreased nitrate, ammonia and Biological oxygen demand (Khademzade et al., 2020).

6.6) Stress tolerance: Supplementation of probiotics significantly improves the stress tolerance of aquaculture species. Stress tolerance in fish to ammonia indicated that fish fed with probiotics *Lactobacillus plantarum* performed a lower increment of cortisol concentration to those of control diet as cortisol is a stress hormone (Nguyen Van Nguyen, 2018). The use of *Aspergillus oryzae* as a probiotic in nile tilapia significantly improve the defence against hypoxia stress (Dawood et al., 2019). Supplementation of dietary commercial probiotics at 0.2% has significantly higher saline water stress tolerance than higher and lower doses in pabda catfish (Chowdhury et al., 2020). This can be concluded from the result that probiotics supplemented groups have lower level of plasma glucose which is the stress indicator in fish.

VII. CONCLUSION

Aquaculture has emerged as a fastest growing industry as it offers a high quality animal protein that supports the nutritional and food security. This growing intensified aquaculture production has various constraints as disease outbreak, high stress condition, shortage of fish meal for protein sources etc . These problems are used to solve traditionally using antibiotics and chemical disinfectants which have raised the questions in food safety to human beings and aquatic animals as their residual effect and finally lead to environmental pollution. In this context, probiotics represents a new era in modern aquaculture as a viable alternative for sustainable aquaculture. Probiotics can be used to establish the favorable condition for the growth of the aquaculture species and hence enhance their health. To enhance the effectiveness of probiotics, the selection of appropriate strain is the most important before its application. Only the effective probiotic at optimum dose has optimum positive impact on particular species.

Among all the routes of probiotics administration, supplementation as feed additives is the most commonly used in aquaculture but application as water additives is the most practically applicable method. The administration via feeding have problem during the early larvae stages due to immature digestive tracts of fish during the larvae stage of development and injection also results in high stress in larvae fish. Hence, addition of probiotics directly in the rearing water is practically applicable in all the stages.

Probiotics enhance the digestive enzyme activities, improves the feed efficiency and hence results in better feed utilization. As we know in intensive aquaculture practices feeding along constitute 60-80 % of operational cost, use of probiotics can manages the feeding cost by enhancing the feed utilization and hence the growth performance. Probiotics application can enhance the health of aquaculture species by increasing the number of useful microorganisms and resist the harmful micro-organisms. Probiotics can also enhance the immune defense mechanism against the fish pathogens. Application of probiotics reduces the environmental and physiological stress in aquaculture species by reducing the level of plasma glucose and cortisol. Probiotics can also enhance the water quality by reducing the level of organic matters and toxic materials.

Although probiotics has beneficial effects on aquaculture management it's uses has been limited in the research and study widely but not used widely at the farm level. The various constraints to probiotics in aquaculture as inability of the strains of aquatic species to be produced in higher quantities, inability of industries to make the probiotic product for aquaculture purposes and hence most terrestrial probiotics are being used in aquaculture.

Keeping in views the various benefits of probiotics application in aquaculture, it can be suggested to the fish farmers and other stakeholders in aquaculture management to incorporate probiotics in feed formulations and use as disease prevention alternative to antibiotics therapy. To widen the use of probiotics at the farm level, the knowledge of administrative method, it's benefits should be provided to the farmer. To reduce the constraints to probiotics in aquaculture the close network of the aquaculture experts, fish nutritionist and microbiologist is necessary. From the above discussion we can conclude that probiotics can be used in the aquaculture widely to enhance the aquaculture production in sustainable way.

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