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Unlocking the potential: A comprehensive review on the application of probiotics in aquaculture

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Abstract
This comprehensive review delves into the multifaceted applications of probiotics in aquaculture, exploring their impact on aquatic health, disease prevention, and overall sustainability. Probiotics, defined as beneficial microorganisms, have emerged as pivotal players in aquacultural ecosystems, offering promising avenues for enhanced productivity and environmental well-being. By unraveling the diverse mechanisms through which probiotics influence fish health, water quality, and disease resistance, this review provides valuable insights for researchers and practitioners. The synthesis of current knowledge aims to optimize the use of probiotics in aquaculture, fostering a deeper understanding of their role in promoting a healthier and more sustainable aquatic environment.

Keywords: Probiotics, aquaculture, aquatic health, disease prevention, sustainability, ecosystem optimization, microbial allies

Introduction
Aquaculture, the fastest growing food production system, is a vital activity, particularly in developing nations, to provide improved food security and better income for poor small-scale farmers. To meet the food demand of the rapidly increasing global human population, the present decade demands the commercialization and intensification of the aquaculture industry. However, the intensification of the aquaculture industry was accompanied by a number of challenges, such as increased stress on the cultured organism and the environment. This can eventually lead to increased susceptibility to disease outbreaks and threats in the aquaculture industry. The use of antibiotics, chemicals, vaccines, etc., has been a common practice as a strategy for the management of fish disease, improving growth and feed conversion efficiency in aquaculture (Suguna, 2020) [23]. The administration of antibiotics in aquaculture has been a general practice among fish farmers for decades; however, it poses a potential risk to the consumers as well as the environment due to their tendency to bioaccumulate in fish muscles. The use of antibiotics as a disease mitigation measure in aquaculture is associated with two main hazards mainly, antimicrobial residues and antimicrobial resistance. As a result of this, the prevalence of antibiotic-resistant microbes increases, creating an imbalance in the gut microflora, thereby affecting fish health and residual deposition in the fish muscle, which will consequently lead to potential health risks to consumers (Subedi *et al*., 2020) [22].
The development of antimicrobial resistance in the fish culture environment can lead to the transmission of antimicrobial resistance genes and bacteria from the aquatic system to the terrestrial environment (to both human and other animal husbandry practices) and vice versa and may result in a detrimental effect on the ecosystem as well as human and animal health (Santos and Ramos, 2018) [20]. Hence, it is the need of the hour to reduce the application of antibiotics and to adopt the mitigation strategies which safeguard the cultured organism, human as well the environment (Reverter *et al*., 2020) [18]. The long-term efforts by various researchers paved the way for the identification of safe, cost-effective and environmentally friendly biotechnological alternatives such as immunostimulants, prebiotics, vaccines, no-specific immune enhancers, probiotics, etc. to reduce the dependence of the aquaculture sector on antibiotics (Suguna, 2020) [23]. Probiotic is an adequate alternative that effectively minimizes the dependence of antibiotics for disease control and at the same time improve

immune response, health, growth, feed utilization and bioremediation of water in a sustainable and eco-friendly manner and hence act as a boon to aquaculture, particularly in view of the organic production system (van Hei *et al*., 2010) [25].

This assignment highlights in brief the probiotics, their type, selection, mode of action, as well as the various beneficial effects of the use of probiotics in aquaculture.

Probiotics and its types

The word "probiotic" is derived from the Greek words, 'pro' means 'for life' and 'biotic' means 'bios' or 'life' (Gismondo *et al*., 1999) [11]. The concept of 'probiotics' was first used by Lilley and Stillwell in 1965, who described these as substances secreted by microbes that stimulate the growth of others (Fuller, 1992) [6]. The word 'probiotic' was first described by Parker (1974) [28], who defined it as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Wang *et al*., 2008) [28]. Later Fuller, in 1989, defined it as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Kesarcodi-Watson *et al*., 2008) [15]. The World Health Organization (WHO) and The Food and Agricultural Organization (FAO) defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Wang *et al*., 2019) [27]. Though the concept and beneficial effect of probiotics had gained immense popularity, its potential application aquaculture as an environment-friendly disease prevention method was adopted by 2008 (Wang *et al*., 2008) [28]. Over time, probiotics have been found to have several applications such as improved fish growth, disease prevention, enhancement of immune response, health status, water quality, sustainability of microbes in the gut, etc., in the aquaculture industry. *Lactobacillus sp*., Lactic acid-producing bacteria, was the first discovered probiotics in aquaculture. Later, a number of other bacteria sp. such as *Aeromonas hydrophila, A. media, Altermonas sp, Bacillus subtilis, Carnobacterium inhibens, Debaryomyces hansenii, Enterococcus faecium, Lactobacillus helveticus, L. plantarum, L. rhamnosus, Micrococcus luteus, Pseudomonas fluorescs* were also found to be effective for potential application in the aquaculture industry (Al-Mamun *et al*., 2018) [2].

There are three types of probiotics used in aquaculture, mainly:

1. **Water probiotics:** The water probiotics are administered directly into the water, either in dry or liquid form, which plays an important role in improving water quality in the culture system (Suguna, 2020) [23]. The water probiotics, when added to the culture medium proliferates, utilize the available nutrients, thereby excluding the pathogenic bacteria via starvation (Hasan *et al*., 2020) [13]. The liquid probiotics exhibit positive response in a very lesser period of time than the dry and spore form though they have a lesser density than liquid form (Suguna, 2020) [23].
2. **Soil probiotics:** The bacteria such as Nitrobacter, Nitrosomonas, sulphur reducing bacteria, etc., are effective in cleaning the aquaculture pond bottom (Suguna, 2020) [23].
3. **Feed/Gut probiotics:** These probiotics are administered orally through the feed and aid in improving the beneficial microbial flora in the gut of fishes (Hasan *et al*., 2020) [13].

Table 1: The various water and gut probiotics used in aquaculture

Probiotics	Beneficial effects
Gut probiotics	
Lactobacillus rhamnosus	Enhance immunity and reduce disease susceptibility
Lactobacillus plantarum	Enhance stress tolerance
Lactobacillus rhamnosus	Improve blood quality
Streptococcus sp.	Improve feeding efficiency and growth rate
Bacillus subtilis	Enhance cellular immunity
Bacillus subtilis + Lactococcus lactis + Saccharomyces cerevisiae	Enhance survival rate, foster metabolism, enhance weight
Bacillus amyloliquefaciens	Enhance antibody concentration, reduce stress
Bacillus subtilis + Lactobacillus rhamnosus	Enhance the food digestibility
Lactobacillus sp.	Reduce pathogen load, provide protection against *Aeromonas hydrophila*
Alcaligenes sp. AFG22	Enhance volatile short chain fatty acids
Different species of Bacillus, Arthrobacter, Paracoccus, Acidovorax etc	Reduce pathogen load and provide nutrients
Bacillus cereu	Protect from *Aeromonas hydrophila* infection
Water probiotics	
Bacillus spp.	Reduces the load of ammonia and nitrite
Enterococcus faecium ZJ4	Improves water quality and enhances immunity
Lactobacillus acidophilus	Improves water quality
Bacillus NL110, *Vibrio* NE1	Reduces ammonia and nitrite concentration
Nitrosomonas sp., Nitrobacters sp.	Reduces the concentration of ammonia, phosphates and nitrite in culture pond
Rhodopseudomonas palustris, Lactobacillus plantarum, Lactobacillus casei, Saccharomyces cerevisiae	Reduces nitrate load, maintain water pH and enhances dissolve oxygen concentration
Paenibacillus polymyxa	Enhances immunity and reduces pathogenic stress
Lactobacillus rhamnosus	Reduces pathogen load in culture tank
Pseudomonas sp.	Enhances transcription rate of anti-microbial peptide
Bacillus spp	Promotes the growth of beneficial algae and reduces the growth of harmful algae
Nitrosomonas sp., Nitrobacters sp.	Reduces pathogen load in culture pond and increases dissolved oxygen content

Source: Hasan *et al*., 2020 [13]

Characteristics of a good probiotics and selection process

The selection of a good probiotic is very crucial as inappropriate selection of stain may result in an undesirable effect on the host species in the system. Therefore, the selection of a safe and effective probiotic strain that provides the desired benefit is important. In addition to this, the selected species should be able to retain their desirable traits during production, manufacturing, distribution and storage. The desirable characteristics of good probiotics are as follows:

1. It should provide the desired effect to the host organism, i.e., Increased growth, improved disease resistance, improved water quality, etc.
2. It should colonize the gastrointestinal tract of the host and should be able to tolerate the lower pH and organic acid in the gut.
3. Non-pathogenic and non-toxic.
4. They should be present as viable cells, preferably in large numbers.
5. Must be stable and remain viable for longer time periods under field conditions and storage.

The flow chart for screening and selection of probiotics in aquaculture is given below (Al-Mamun *et al*., 2018) [2]:

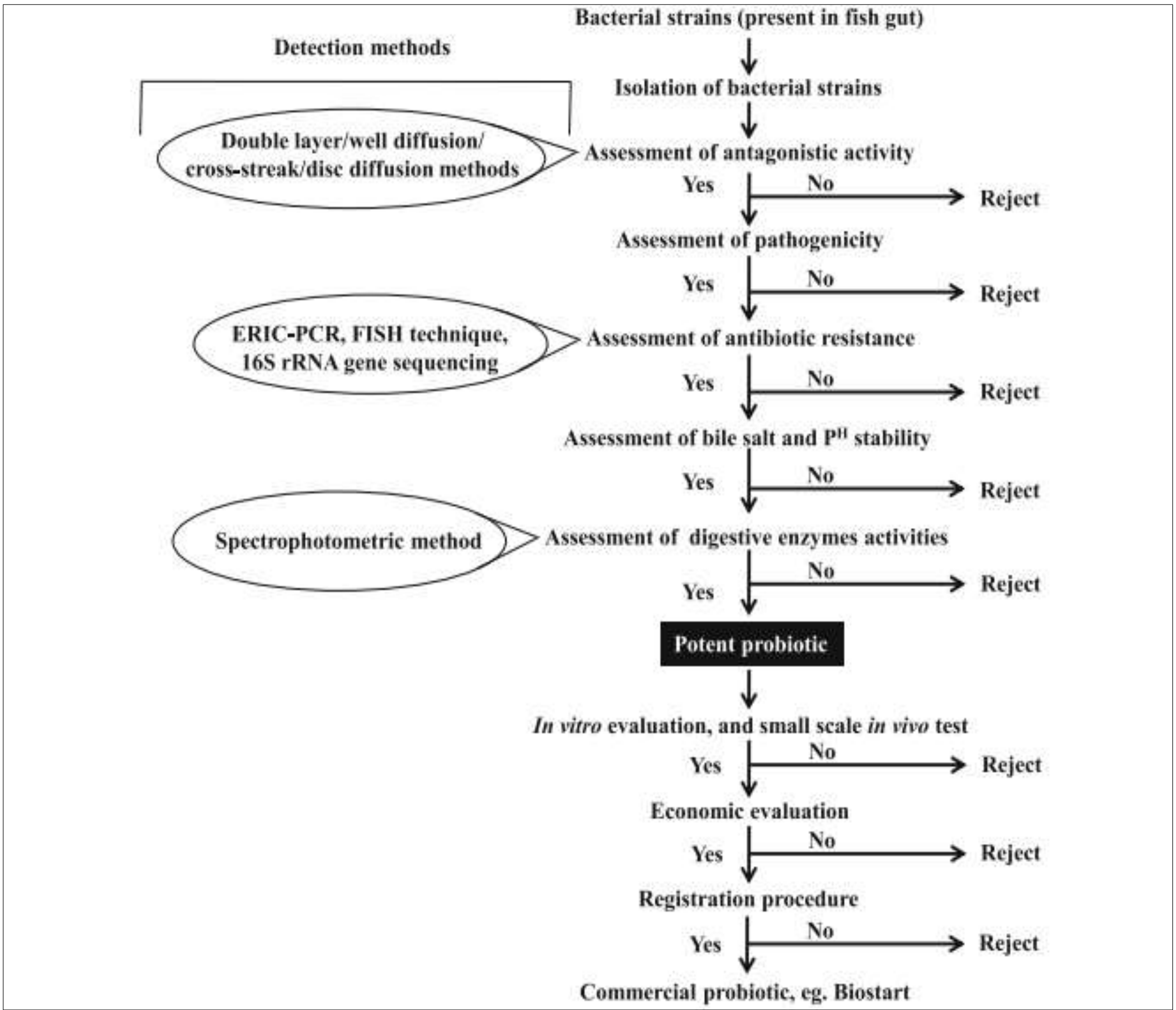
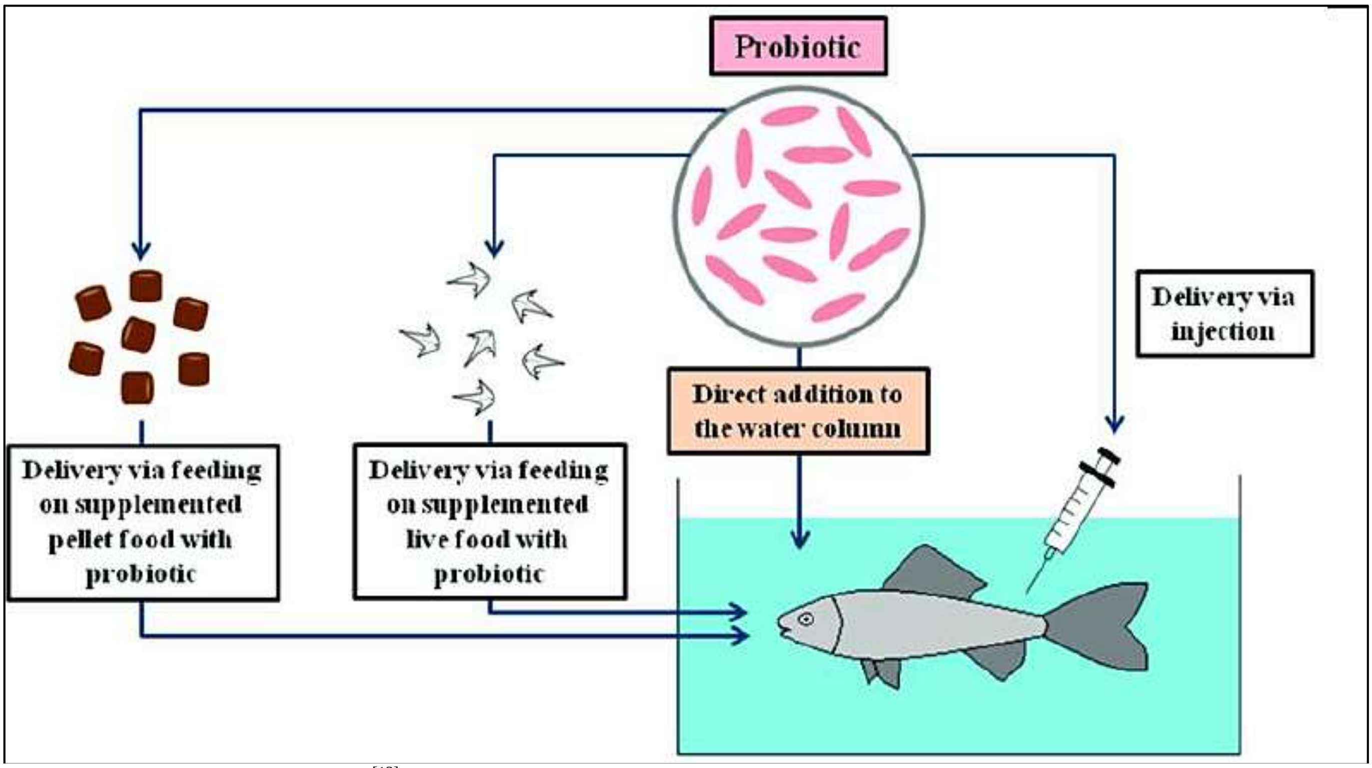


Fig 1: Flow chart for screening and selection of probiotics in aquaculture

Mechanism of probiotic action

Probiotics suppress the growth of harmful bacteria and promote the growth of intestinal microbiota in the gut of fishes and thereby strengthening the natural defence mechanism of fish's body and thus improving the resistance against infectious diseases (Giraffa *et al*. 2010; C. De *et al*. 2014; Gildberg *et al*. 1997) [10, 4, 9]. The bacterial probiotics do not exhibit any mode of action and respond via species-specific, strain-specific immune responses of the fish and interaction with intestinal bacterial communities (Simon, 2010) [21]. The mode of action of probiotics is through the production of inhibitory substances, which act as antagonistic to the pathogens in the stomach and thus prevent their growth. Some probiotics may also adhere to the intestinal mucus and hence blocks the intestinal infection route of pathogens in the gut (Ringo *et al*. 2010; Gatesoupe, 1999) [19, 7]. In addition to the inhibitory action of the probiotics on the pathogenic bacteria, these probiotics may also flourish on the gut and hence stimulate the appetite and improve the nutrition of host through the production of vitamins, detoxification of toxic compounds in diet and also enhance the breakdown of indigestible compounds in the gut (Abdelhamid *et al*. 2009; C.De *et al*. 2014) [1, 4].

Method of administration of probiotic



(**Source:** Jahangir and Esteban, 2018) [13]

Fig 2: Different routes for administration of probiotic

The probiotics can be administered via various routes such as injection, through fish feed or via direct immersion of probiotics in water and this can be provided either using a single strain or through administration of various probiotic strains in combination. Another major consideration in the administration of probiotics is the dosage of application, which depends on the probiotic species used, the fish species, its physiological status and the goal of the probiotic application. Hence, the dosage of probiotic application is a crucial factor while supplementing along with aquafeed as higher concentration showed no positive impact on fish growth and physiology and may result in disruption of carbohydrate and fat metabolism in fish (Subedi *et al*.,2020) [22].

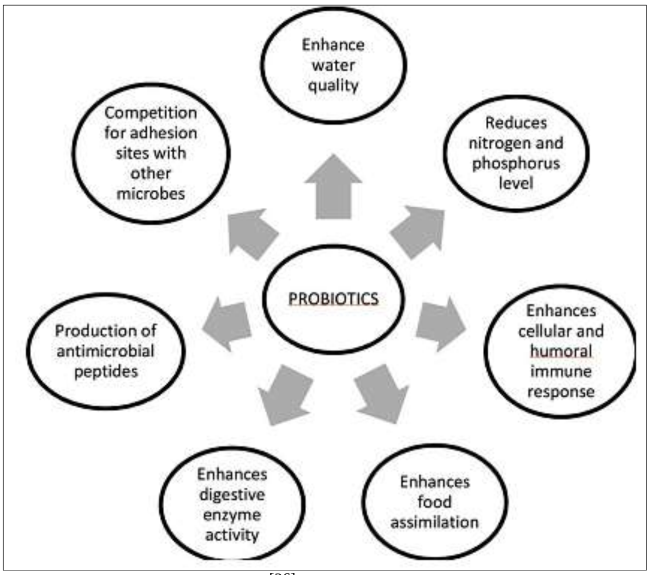
a) Feed additive, water additive and injection

The most widely used method of administration of probiotics in aquaculture is via the incorporation in the fish feed (92.8%), followed by addition to water (4.8%), incorporation in live food (1.6%), while the method of injection is less popular (Melo *et al*., 2020) [15]. The bacterial, yeast and other substances are commonly administered via feed in aquaculture either through direct incorporation in the feeding pellet, encapsulation in live feed or administered orally along with feed to the cultured organism. These probiotics as feed additives such as *Lactobacillus plantaram, Bifidobacterium* strain, etc., were found to provide various benefits such as improved growth performance, enhancement of non-specific immunity, nutrient utilization, etc. The water additives like *Vibrio lentus* significantly improved the gene expression in fish through enhanced immune response, cell proliferation, adhesion of dead cells, iron transport, etc. The use of the injection method for the administration of probiotics is less common. The injection is provided either via the intramuscular or intraperitoneal route of Enterobacter strain provides enhanced immunity to fish (Subedi *et al*., 2020) [22].

b) Administration of probiotics singly or in combination

The probiotics in aquaculture were administered either singly or in combination, such as multiple strains of probiotics or probiotics along with plant extract or yeast extract, etc. The various studies on the probiotic application in aquaculture were mainly focused on the use of a single probiotic, while the use of a combination of probiotics is reported to be more effective (Subedi *et al*., 2020) [22]. The multi-strain of probiotics is more effective as they are more sensitive towards pathogens and are more active against various aquaculture organisms (Pannu *et al*., 2014) [17]. Van Doan *et al*. (2018) [24] reported that the combined use of *Lactobacillus plantarum* N11 and *Bacillus velezensis* H3.1 in *Oreochromis niloticus* enhanced the higher survival rate of 58.33% as compared to a singly application of *Lactobacillus plantarum and Bacillus velezensis* which exhibited a survival rate of 54.17% and 41.67%, respectively. Hamka *et al*. (2020) [12] reported that the use of a combination of *Bacillus megaterium* PTB 1.4 and *Pediococcus pentosaceus* E2211 through a fish feed provided better performance than the single use of either of the two probiotics. The combined use of probiotics (*Bacillus coagulans) and* plant extract (*Mentha piperita)* ensured better growth performance, nutrient retention and immunity in Catla as compared to a singly application (Bhatnagar and Saluja, 2019) [3].

Beneficial effect or application of probiotics in aquaculture



Source: Verma *et al*., 2015 [26]

a) Improved feed utilization and growth performance of fish

The use of probiotics causes alteration of enzymes and thus contribute to improved feed utilization by fish. The probiotics also affect the growth performance of fish via improved nutrient uptake by fish or by providing an increased amount of nutrients for gut probionts to grow in the gut of fish (Subedi *et al*., 2020) [22]. *Bacillus sp*. is the most widely used bacterial probiotic in aquaculture, which are administered through feed supplement and thus provide enhanced growth and also improve feed utilization via digestive enzyme enhancement through increased digestion of protein, starch, fat, etc., thus helping in better growth and survival. Probiotics are widely used in the shrimp industry as growth promoters and also increase growth performance such as weight gain, specific growth rate, survival, relative per cent survival and feed utilization in terms of food conversion ratio and protein efficiency ratio (Verma *et al*., 2015) [26].

b) Increased disease resistance

The probiotics usage assists the organism in attaining natural resistance and thus controlling fish diseases. The probiotic microbes release chemicals having a bactericidal or bacteriostatic effect on the pathogenic organism in the host intestine, thereby competing with them, eliminating and thus providing enhanced disease resistance (Subedi *et al*., 2020) [22].

c) Enhancement of the immune response:

Probiotics also enhance the immunological parameters of a host organism by enhancing the host immune system via non-specific and cellular immunity. These probiotics enhance cellular and humoral immunity, increase lysozyme activity, respiratory burst activity, reduces superoxide dismutase (SOD) assay, increase red blood cells (RBC), white blood cells (WBC), haematocrit, total serum antibody level, alternative complement, protease, and antibacterial activity, etc. in fish and thus enhance the immune response in the cultured organism (Verma *et al*., 2015; Subedi *et al*., 2020) [26, 22].

Eg. *Lactococcus lactis, Lactobacillus sakei, Lactobacillus plantarum, Bacillus velezensis, Saccharomyces cerevisiae,* etc.

d) Improve water quality

The improvement of water quality can also be achieved via the use of water probiotics as these bacteria have ability to directly uptake or aid in decomposition of organic matter and toxic compounds from water resulting in enhanced water quality in the system (Subedi *et al*., 2020) [22]. Probiotics act on the organic matter and decompose the fish excreta, food remains and other organic and inorganic matters like carbon dioxide, nitrogen, ammonia, nitrite, phosphate, etc. and hence improving the nutrient cycling and thus improving the water quality of cultured environment. The most widely used probiotic for water remediation is the *Bacillus* species, as these are stable for a long period due to spore formation and can be easily prepared by fermentation and due to their antagonistic effect on pathogenic bacteria (Verma *et al*., 2015) [26]. The commonly used bacterial water remediators includes *Bacillus amyloliquefaciens, Lactobacillus* strains, *Bacillus cereus, Pediococcus acidilactici, Lactobacillus plantarum*, *Aspergillus oryzae, etc.* (Subedi *et al*., 2020; Verma *et al*., 2015) [22, 26].

e) Stress tolerance

The use of probiotics in aquaculture also improves the stress tolerance of cultured organism. *Lactobacillus plantarum* improves the tolerance of fish against ammonia toxicity, lowers cortisol concentration, plasma glucose, etc. in fish and thus improve the stress tolerance of cultured species in aquaculture. Commonly used stress-tolerant probiotics includes *Lactobacillus plantarum*, *Aspergillus oryzae, etc.* (Verma *et al*., 2015) [26].

Conclusion

Aquaculture has played a distinct role in the development of the socio-economic condition of our country. Though intensification is considered a boon for the sector, it's necessary to address the possible challenges of intensification associated with various use of drugs and chemicals, especially antibiotics, as well as the use of the organic approach for environmental bioremediation and disease control in the system. Probiotics serve as biological control of disease via environmental friendly and sustainable methods for reducing stress and diseases, healthy gut environment, the health of aquatic animals, improved water quality parameters, higher survival, growth, production, productivity, etc. in aquaculture. Hence, the use of probiotics ensures the successful management of organisms and the environment as well as human health.

References

1. Abdelhamid AM, Mehrim AI, El-Barbary MI, Ibrahim SM, Abd El-Wahab AI. Evaluation of a new Egyptian probiotic by African catfish fingerlings. J Environ Sci Technol. 2009;2(3):133-145.
2. Al Mamun MA, Nasren S, Bari SM. Role of probiotics in aquaculture: Importance and future guidelines. J Bangladesh Acad Sci. 2018;42(1):105-109.
3. Bhatnagar A, Saluja S. Synergistic effects of autochthonous probiotic bacterium and Mentha piperita diets in *Catla catla* (Hamilton, 1822) for enhanced

growth and immune response. Fish Aquat Sci. 2019;22(1):1-14.
4. C De B, Meena DK, Behera BK, Das P, Das Mohapatra PK, Sharma AP. Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses. Fish Physiol Biochem. 2014;40(3):921-971.
5. El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MA, *et al*. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol. 2021;117:36-52.
6. Fuller R. History and development of probiotics. In: Probiotics. Dordrecht: Springer; 1992. p. 1-8.
7. Gatesoupe FJ. The use of probiotics in aquaculture. Aquaculture. 1999;180(1-2):147-165.
8. Gildberg A, Mikkelsen H, Sandaker E, Ringø E. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). Hydrobiologia. 1997;352(1):279-285.
9. Giraffa G, Chanishvili N, Widyastuti Y. Importance of lactobacilli in food and feed biotechnology. Res Microbiol. 2010;161(6):480-487.
10. Gismondo MR, Drago L, Lombardi A. Review of probiotics available to modify gastrointestinal flora. Int J Antimicrob Agents. 1999;12(4):287-292.
11. Hamka MS, Meryandini A. Growth performance and immune response of catfish Clarias sp. given probiotics Bacillus megaterium PTB 1.4 and Pediococcus pentosaceus E2211. Jurnal Akuakultur Indonesia. 2020;19(1):50-60.
12. Hasan KN, Banerjee G. Recent studies on probiotics as beneficial mediator in aquaculture: A review. J Basic Appl Zool. 2020;81(1):1-16.
13. Jahangiri L, Esteban MÁ. Administration of probiotics in the water in finfish aquaculture systems: a review. Fishes. 2018;3(3):33.
14. Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture. 2008;274(1):1-14.
15. Melo-Bolívar JF, Ruiz-Pardo RY, Hume ME, Sidjabat HE, Villamil-Diaz LM. Probiotics for cultured freshwater fish. Microbiol Aust. 2020;41(2):105-108.
16. Pannu R, Dahiya S, Sabhlok VP, Kumar D, Sarsar V, Gahlawat SK. Effect of probiotics, antibiotics and herbal extracts against fish bacterial pathogens. Ecotoxicol Environ Contam. 2014;9(1):13-20.
17. Reverter M, Sarter S, Caruso D, Avarre JC, Combe M, Pepey E, *et al*. Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat Commun. 2020;11(1):1-8.
18. Ringo E, Løvmo L, Kristiansen M, Bakken Y, Salinas I, Myklebust R, *et al*. Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: A review. Aquaculture Res. 2010;41(4):451-467.
19. Santos L, Ramos F. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. Int J Antimicrob Agents. 2018;52(2):135-143.
20. Simon O. An interdisciplinary study on the mode of action of probiotics in pigs. J Anim Feed Sci. 2010;19:230–243.
21. Subedi B, Shrestha A. A review: Application of probiotics in aquaculture. Int J Forest, Animal Fisheries Res. 2020;4(5):52-60.
22. Suguna T. Role of Probiotics in Aquaculture. Int J Curr Microbiol Appl Sci. 2020;9(10):143–149.
23. Doan VH, Hoseinifar SH, Khanongnuch C, Kanpiengjai A, Unban K, Srichaiyo S. Host-associated probiotics boosted mucosal and serum immunity, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). Aquaculture. 2018;491:94-100.
24. Hai VN, Fotedar R. A review of probiotics in shrimp aquaculture. J Appl Aquac. 2010;22(3):251-266.
25. Verma G, Gupta A. Probiotics application in aquaculture: improving nutrition and health. J Anim Feed Sci Tech. 2015;3:53-64.
26. Wang A, Ran C, Wang Y, Zhang Z, Ding Q, Yang Y, *et al*. Use of probiotics in aquaculture of China—a review of the past decade. Fish Shellfish Immunol. 2019;86:734-755.
27. Wang YB, Li JR, Lin J. Probiotics in aquaculture: challenges and outlook. Aquaculture. 2008;281(1-4):1-4.
28. Parker GA. Assessment strategy and the evolution of fighting behaviour. Journal of theoretical Biology. 1974 Sep 1;47(1):223-243.