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Advances in probiotic research for aquaculture: screening methods, mechanisms, and regulatory considerations

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Aquaculture's swift rise to prominence as the world's main source of animal protein has created problems with disease outbreaks, antibiotic resistance, declining water quality, and degradation in environmental sustainability. Probiotic strains such as *Bacillus, Lactobacillus, Enterococcus*, and *Pseudomonas* exhibit notable capability to enhance stress tolerance, immunity, host growth, and reproductive efficiency. Critical evaluation parameters to guarantee safety and effectiveness, including hemolysis assays, antibiotic susceptibility testing, bile and pH tolerance, adhesion and colonization ability and pathogen antagonism. The paper emphasizes how probiotics improve hematological indices, antioxidant status, feed utilization, digestive enzyme function, and resistance to common fish infections like Aeromonas. Probiotics also help to improve water quality by reducing hydrogen sulfide and ammonia, cycling nitrogen, and lowering chemical and biological oxygen demands. Despite encouraging results, various issues with strain standardization, long-term biosafety evaluation, horizontal gene transfer tracking, and species dosage optimization were noticed.

Keywords: Fish gut, Probiotics, Prebiotics, Water quality

Introduction

The world's population is increasing day by day, and various problems like scarce resources and restricted access to food[1]. Aquaculture has become a crucial requirement for a cheap, high-protein food supply, improving nutritional safety and supporting many trades internationally[2]. The food production industry is experiencing rapid growth; in 2022, international manufacturing hit 130.8 million tons, making it the biggest industry in the international food production trade[3].

Although essential, the fish farming industry is nevertheless facing obstacles that jeopardise its long-term sustainability[4]. Among such problems are the pandemic, ineffective use of food, declining water purity, and misuse of antibiotics, that is increase effective expenses[5]. These challenges lead to significant financial losses. That is especially appropriate in more developing nations, like Egypt, Vietnam, and India, they are important producers of fish raised for food, but frequently employ semi-intensive conventional farming techniques[6]. Due to the absence of understanding and current technology supporting sustainable practices, these processes are vulnerable to outside influences and potential output losses, which is crucial to ansuring the industry's long-term viability[7].

One viable method for addressing these problems is the use of probiotics. Probiotics may be a type of good microorganisms that fight prevalent illnesses in freshwater, such as *Aeromonas* and *Streptococcus* species[8]. Through the development of antibacterial substances that stop bacteria from growing, besides competing for food and space, they combat illness[9]. Additionally, probiotics reinforce the intestinal barrier, improve the host's immune

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response, and increase feed efficiency[10]. By maintaining a healthy microbial balance, they are decreasing, which is a significant source of death in farmed fish[11]. Finally, give a comprehensive summary of antibiotics, including their origin, isolation, selection standards, advantages, and mechanisms for use in aquaculture[12]. It does this by offering crucial statistics or a strong foundation to study for the purpose of producing more environmentally friendly solutions that enhance the health of fish, enhance the quality of the water, and lower expenses for the manufacturing of the aquaculture industry[13,14].

Source of probiotics for aquaculture and associated research gaps

It is possible to isolate beneficial bacteria from a variety of environments, such as aquatic creatures, natural and clean aquatic water columns, soil, silt, rainwater, and additionally humans[15]. That indicates all ecosystems evidently contain microorganisms. Different types of bacteria colonize the gastrointestinal tract of animals regardless of the source of isolation; this occurs when microbes from outside the body (from human, soil, or atmospheric sources) pass into aquatic environments and then colonize populations of microorganisms[16,17]. Examples of microorganisms with potential uses in aquaculture that were isolated from various sources, including sediments, water, fish guts and soil,[18] are listed in Table 1. This demonstrates that probiotics with potential for success originate from various sources, not only aquatic ones, and that investigating unconventional sources may yield new strains that are most advantageous to aquaculture[19]. The absence of defined procedures for separating and screening these many microbial sources, however, is a crucial gap in this field that makes it challenging to compare finding from various investigations[20].

Probiotic selection and efficacy evaluation

The following criteria would be considered when assessing the possible effectiveness of probiotics for an aquatic species:

In vitro research eliminates the need for live animals and provides an affordable substitute for in vivo tests for assessing the potential efficacy of a novel probiotic strain[28,29]. Pathogen antagonism assay is a popular screening technique for determining probiotic activity. Nevertheless, this criterion is not the only one that might be helpful. Several researches have used different option procedures regarding probiotics, such as patterns of blood hemolysis, antibiotic susceptibility, adhesion tests, tolerance to bile salts and pH, and their impact on the development of the host and immunology[21]. Some prioritize evaluating pathogenic activity before doing safety tests, such as hemolysis and pathogen inhibition. Adhesion characteristics, pathogenic inhibition, and bacterial aggregation have all been studied by others[30,31].

Although experimental methods are valuable, they frequently cannot reproduce the intricate relationships observed in a living host's intestines, that causes an important disparity between lab results and field effectiveness. Therefore, an additional in vivo investigation is essential to verify the suitability of the suggested strains. Safety evaluations that examine synthesis of hemolysin, susceptibility to antibiotics, salt deconjugation of bile, breakdown of mucin, enzymatic activity, and the existence of genes that resist antibiotics are used to identify probiotics[32]. Furthermore, studies have indicated that isolates with distinctive traits, like surface of the cell features, automatically aggregate using recognised aquaculture infections, and adherence into the host gut and various substrates, may be successful[33].

Hemolysis assay as a safety standard for aquatic probiotic strains

The blood hemolysis test is an essential part of assessing a probiotic strain's safety because it measures its hemolytic function, which is significant component of pathogenicity[34]. The test's ability to lyse red blood cells is evaluated by cultivating it on blood agar. Three categories of hemolysis: Alpha (greenish discolouration and partial hemolysis), Gamma (absence of hemolysis) and Beta (complete hemolysis with a zone of clarity)[35]. Since beta hemolysis is frequently linked to pathogenic bacteria. This test strengthens the candidate probiotic's suitability for aquaculture applications by confirming that it does not endanger red blood cells. Table 2, distinguishes between fish associated microorganism known for their hemolytic tendencies in relation to their putative probiotic qualities[23]. It should be noted, nevertheless, that many commensal bacteria that are not harmful may exhibit mild alpha or even weak beta hemolysis, making it difficult to interpret this one test for complete safety.

Antibiotic susceptibility and resistance risk in aquatic farming probiotics

Compounds and metabolites that inhibit or impede the growth of other microbes are naturally produced by bacteria[38]. Natural competition and ongoing evolutionary processes are the causes of this extraordinary behaviour. As a result, antibiotic resistance genes (ARGs) may spread horizontally to different bacterial strains or species as well as vertically within the same species[39]. Additionally, some research discovered that certain *bacillus species* and other probiotic bacteria are inherently resistant to the medications, *penicillin and kanamycin*[40]. Testing for resistance is important, but it doesn't ensure that other resistance mechanisms, such as ARGs. Similar to other bacteria, probiotics are able to take the resistance genes from their surroundings once they are introduced

Table 1. The characteristics and source of isolation of the probiotic strain

Strain of probiotics	Reason for isolation	Desired species	Properties of probiotics	References
Enterococcus, Lactococcus	The gut of freshwater fish	*Puntius filamentosus, Channa striata, Oreochromis mossambicus, Cirrhinus mrigala,* and *Rasbora daniconius*	Production of enzymes	21
Bacillus subtilis, B. velezensis, Priestia flexa, Cytobacillus firmus	Sediment, shrimp, and lake water	*Litopenaeus vannamei, Ctenopharyngodon idella*	Activity of enzymes	22
Lactic acid bacteria: Lactococcus, Enterococcus, Lactobacillus, Weissella	Intestinal tract of fish	Marine & freshwater fish	The antimicrobial properties Bile/acid tolerance Secretion of enzymes	23
Bacillus spp. (B. subtilis, B. velezensis, B. amyloliquefaciens)	Aquatic habitats and the gut of fish	Both freshwater and saltwater fish	Formation of spores The synthesis of enzyme Pathogen management Tolerance of stress	24
Bacillus subtilis, B. cereus, B. amyloliquefaciens	Digestive tract of fish	*Labeo calbasu*	Activity of enzymes Tolerance to acid/bile	25
Bacillus rugosus NM007	Soil	*Nile Tilapia*	The formation of pores Enzyme production Pathogen management	26
Bacillus spp.	Water, Sand and the GI Tract	Shellfish, Finfish,	Enzyme activity Antimicrobial action	27
Lactobacillus, Enterococcus, Bacillus sp.,	Gastrointestinal tract, water	Shellfish	The synthesis of enzyme Immunomodulation	25

Table 2. Bacterial species' hemolytic qualities and appropriateness as probiotics

Type of hemolysis	Description	Probiotic safety	Examples	References
Beta(β)	Total hemolysis; the colony's surrounding area is clear	Unsafe	*Bacillus pseudomycoides, Bacillus safensis, B. infantis*	36
Gamma(γ)	Blood agar changes; no hemolysis	ot safe	*Lactobacillus* spp., *Enterococcus spp.* and some *Staphylococcus* spp.	37
Alpha(α)	Hemolysis in part; Greenish discoloration	Safe under certain conditions	*Bacillus infantis*	36

to a farm. The long-term , secure usage of probiotics in aquaculture is constantly threatened by the possibility of the development of resistance mechanisms in both harmful and beneficial microbes[41]. Despite this risk, a substantial biosafety issue remains unresolved because the majority of commercial probiotic products are not subjected to thorough, long-term monitoring of ARG transfer in actual farm contexts[42].

Adherence and colonization assays for the effectiveness of probiotics

A probiotic strain's probiotic potential depends on its ability to stick with the surfaces of intestines, assemble and effectively take over the host[43]. It outcompetes pathogens, increases its persistence in the gut, and promotes advantageous interactions with the host when it adheres to the intestinal mucosa. Biofilm development and the competitive exclusion of pathogenic microorganisms are facilitated by auto-aggregation and co- aggregation features[44]. Long- term probiotic advantages, such as better nutrient absorption, immunological modulation, and gut health, are guaranteed by successful gut colonization[45]. Assessing these traits aids in determining the strain's capacity to infiltrate the host and generate its advantageous effects on aquaculture[46,47].

Probiotic strain selection using pH and bile tolerance

When choosing probiotic strains, one of the most crucial characteristics its capacity to tolerate variety of bile acid concentrations and pH, this permits them to endure also continue to function within the digestive system[48]. A bacterial strain must be able to withstand severe conditions of gastrointestinal, particularly bile salts in the intestine and a low pH in the stomach, to be deemed an efficient probiotic in aquaculture, for strains of probiotics meant therefore, bile content (usually 0.3-0.5%) and acid tolerance (pH 2-3) are important selection factors for oral administration to fish[49,50]. While the concentration of bile, 0.3-0.5%, is used as a threshold for *in vitro* probiotic screening, different fish species may have different real bile concentrations[51]. Thus, when defining tolerance criteria, species-specific physiological facts should be taken into account. Furthermore, the bacteria exhibit promising survival and accessibility outcomes at reduced levels in a typical gut setting due to their capacity to endure at greater concentrations of bile salt in vitro, and it is not typically found in fish guts in such high proportions.

Antioxidants antagonistic impact on fish infections

In aquaculture, its antagonistic effect on pathogens is an important quality of feasible probiotic candidates[52]. This effect illustrates how probiotics may prevent or reduce the virulence of harmful microorganisms that cause fish diseases[53]. It is crucial for maintaining the aquatic environment and fish digestive systems[54]. Numerous mechanisms contribute to this conflict of interest, including the synthesis of antimicrobial compounds that directly stop the growth of infections, such as hydrogen peroxide, organic acids, and bacteriocins[55]. By diseases for reasons of attachment to the gut's mucosal membranes, probiotics may also stop colonization and infection. By decreasing the likelihood that harmful microbes would colonize and cause diseases, this process of competitive exclusion fosters a more robust and balanced host microbiome[56].

Additionally, a variety of probiotic strains may outcompete dangerous microbes are important for vital nutrients, especially in surroundings with minimum resources[57]. Probiotics indirectly suppress the proliferation of harmful microbes by depriving them of essential nutrients required for their growth and survival. Strong inhibitory effects against regular fish infections, includes *Aeromonas hydrophila, Vibrio anguillarum, Edwardsiella tarda, and Streptococcus iniae* have been shown by several strains, particularly those from the genera *Bacillus, Lactobacillus,* and *Pseudomonas*[58]. Applying these kinds of probiotics to nourish fish or water, they may dramatically lower the frequency and severity of infections brought on by the pathogens, according to laboratory and *vivo* research[59].

Application, mechanism, and mode of probiotics in aquaculture

Recent studies in aquaculture has focused on the importance of using probiotics, that is clearly beneficial for the overall health of fish, increasing growth and reducing sickness while also enhancing gut and blood health through several processes[60].

Impact of probiotics on growth performance and feed efficiency

Like all living things, fish farming relies heavily on nutrition to support growth and production[61]. Frequencies of growth, digestion, nourishment use, and cost are all impacted by probiotic use[62,63]. The growth promoters' existence and other significant effector metabolites, including hydrolytic enzymes, which aid fish in optimizing the nutritional advantages, have a good effect on growth rates[64,65]. For example, *Lactobacillus acidophilus, streptococcus faecium, Lactobacillus fermentum, Saccharomyces cerevisiae,* and other *Bacillus species* are helpful bacteria that support fish growth[66,67].

Probiotic supplementation is already demonstrated to improve the biological availability and activity of many vitamins, such as "cobalamin (B12), biotin (B7), and vitamin K. *Bacillus spp."* Probiotics, like "*Bacillus licheniformis",* expand feed conversion ratio (FCR), specific growth rate (SGR), and protein efficiency ratio (PER) in a 2024 study[68,69,70]. Additionally, they optimize the advantages of small-protein diets, which may reduce production expenses. It also improves fish growth performance by increasing nutrient availability and digestibility[71].

To meet the increasing demand for fish around the world, probiotics aid in shortening the harvest season then increasing production efficiency. Probiotics are important in semi-intensive tilapia production systems was discussed in study[53,72]. According to the study's findings, adding probiotics to water improves its quality, increases growth, survival, and productivity of fish. *Bacillus subtilis* in the bio-floc system do not significantly increase the expansion rate of *Litopenaeus van-namei,* according to study[73]. However, when the addition strategies were compared, it became apparent that using probiotics into the meal worked better than putting them in the water because it improved the quality of the water, activity of intestinal digestive enzymes, and also the activity of divergent immune enzymes. *Nila tilapia* fed probiotics had ultimate weights that were up to 30% greater than those of the control group, according to a study[74].

It is stated that probiotics showed the enormous freshwater shrimp *Macrobrachium rosenbergii* has enlarged digestive activity following the consumption of *Bacillus coagulans,* that rises the absorption and development of digestive enzymes, specifically protease, amylase and lipase[75,25]. The greatest protein efficiency, FBW, WG, and SGR were noted if *Mugil capito* fish was administered with a probiotic blend of *Saccharomyces cerevisiae and Lactobacillus bulgaricus*[76]. Additionally, "*Nile tilapia"* were nourished diets that contained *ruminococcus flavefaciens,* serum globulin, serum glucose, albumin, cortisol levels, and protein levels overall increased. In European sea bass, the same probiotic-containing diet in SGR, *Ruminococcus flavefaciens,* improved while the body composition in terms of ash, dry matter, and crude protein remained unchanged. When European Sea Bass (Dicentrarchus labrax L.) are fed *Pediococcus acidilactici* as probiotics, they grow taller[77,78]. Despite these encouraging results, more transcriptome and proteomic research is required because the molecular pathways by that probiotics enhance food utilization are frequently suggested slightly than straight assessed.

Impact on resilience to stress and health

Probiotic gradually been acknowledged for its effects on bolstering the host's resistance to illness and reducing the detrimental effects of numerous stimuli, in addition to their established role in promoting digestive health[79].

Effects of probiotics on fish liver health and antioxidant activity

Probiotics interventions raise albumin, globulin, and protein while lowering AST and ALT[80]. They also improve the activities of glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). The animal body's resistance to oxidative stress is strengthened by these modifications[81,82]. Through processes such as ion chelation, reactive oxygen species reduction, oxidant secretion inhibition, and ascorbate autoxidation reduction, they have an antioxidant impact[83].

In comparison to the control group, the probiotic-treated groups' histological analysis of hepato-pancreatic tissue revealed melanocyte centres, enhanced glycogen storage, and well – organized parenchyma. Although there are clear histological improvements, further research is required to connect these morphological alterations to particular metabolic and physiological processes that encourage improved liver function[84].

Probiotics' effects on blood health, illness resistance, and fish immunity

Fish are surrounded by a variety of aquatic pathogens, so when the fish's immune system deteriorates, and then bacteria locate an ideal setting, they able to take benefit and spread illness[85]. The fish's resistance to illness was enhanced by probiotics[86]. When fed a probiotic supplement, fish show no clinical symptoms or expiration when challenged with *Streptococcus iniae*[87]. As per previously stated, certain probiotics, like *Enterococcus faecium*, like *Enterococcus faecium,* may produce vitamins like B7 and B12 and enhance durability. They may also raise haemoglobin, hematocrit, red blood cells, and white blood cells, which enhances immunity, disease resistance and effectively combat certain infections like *Aeromonas hydrophila.* Additionally, probiotics may boost humoral and cellular immunity[88,89]. Additionally, fish given a diet containing *Bacillus coagulans* exhibit improved respiration, phagocytic activity, lysozyme activity, and white blood cell counts. Better growth, enhanced immunity, and efficacy against *Aeromonas hydrophila* are the outcomes of this[90].

Fish given a meal that contains a single-strain of probiotic or a combination of multiple strains of probiotics with reduced levels of cholesterol (T-Cho), glucose (GLU), aspartate aminotransferase (AST), total glycerides (TG), and alanine aminotransferase (ALT)[53]. The variations in blood urea nitrogen (BUN), total protein (T-Pro), hemoglobin (Hb), total bilirubin (T-bill), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). "*Bacillus amyloliquefaciens, Lactobacillus plantarum,* and *Bacillus mesentericus"* strains were used either alone or in combination. The results indicated that *Bacillus amyloliquefaciens* performed well when combined with other bacteria than when used alone[53] (Fig. 1).

The *bacillus* coagulant strain may increase white blood cells (WBCs), heterophils, and neutrophil count in hematology (NCH), and neutrophil-to-lymphocyte count ratio (NCHC), demonstrating the impact of these strains on fish immunological function[91]. In addition to increasing red and white blood cells, probiotics also positively impact lysozyme and phagocytosis activity, which enhances fish immunity to illness[92,93]. More strains are being discovered as research progresses, and the strain *B. rugosus NM007* exhibits an excessive percentage of both *Aeromonas and Streptococcus sp*. co-occurrence[94]. The capacity to develop probiotics with specific immunomodulatory effects is limited since precise method by which certain probiotic strains stimulate particular immunological routes in various fish species remains undiscovered, despite these promising discoveries.

Impact on fish reproduction and hormonal regulation

Viability of fish aquaculture depends on effective and dependable superior reproductive process is also reproduction. The presence and efficacy of hormones affect

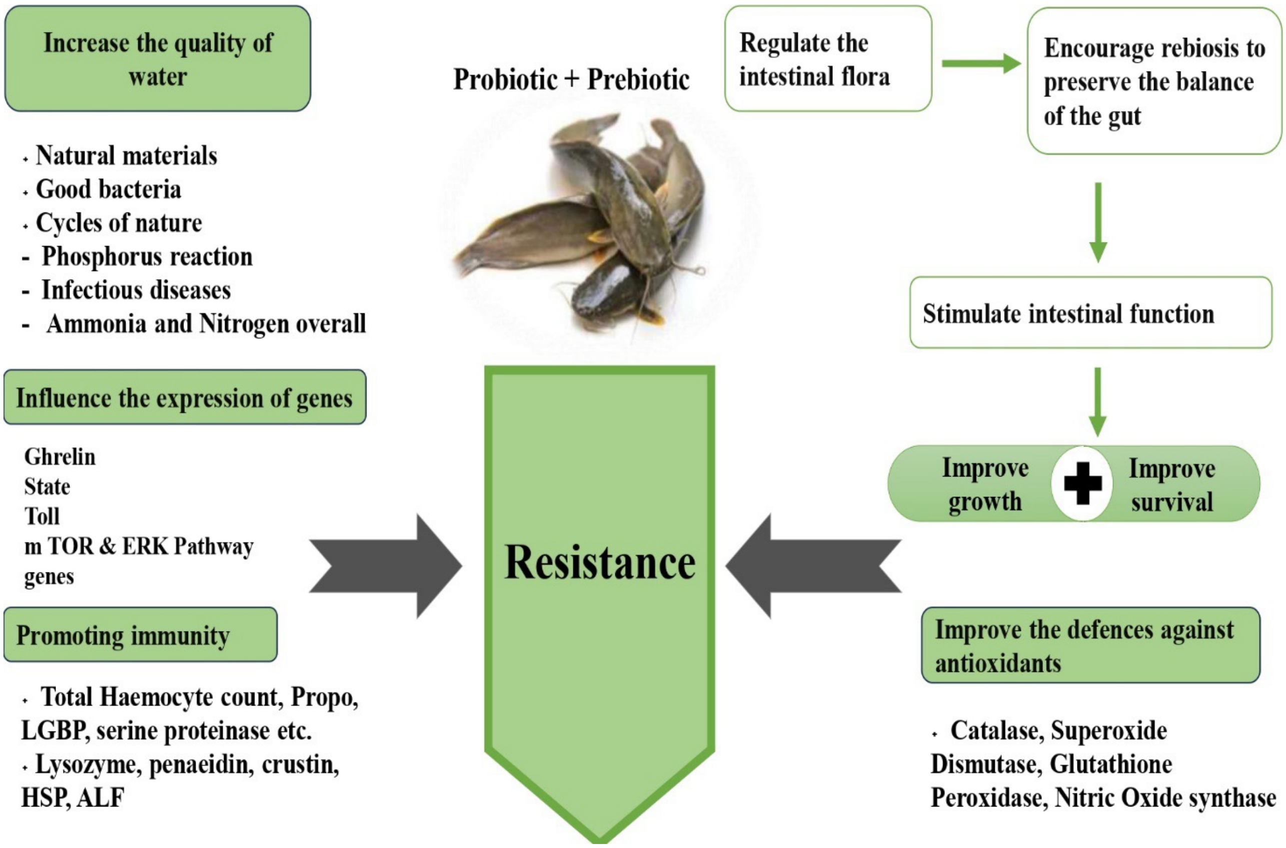

Figure 1. The effects of probiotics on the physiological functioning of fish and health

the quality of reproduction, and the amounts of hormones fluctuate according to the maturity stage of fish[95,96]. It has been shown that the consumption of probiotics in fish, particularly tilapia, zebrafish, rainbow trout, catfish, and goldfish, enhances the synthesis of important reproductive hormones such as progesterone, FSH, LH, testosterone, and estrogen. That is achieved by strengthening genes connected to the kisspeptin/GnRH/GTH axis, and they are necessary for regulating fish reproduction. All probiotic strains and concentrations consistently demonstrate enhanced hormone levels, with higher doses frequently yielding higher advantages[97,98]. The absence of enduring investigations monitoring the consequences of probiotic supplements on the health of farmed fish reproduction, however, represents a significant gap in the current body of research.

Role of bacteria in enhancing water quality

The most crucial elements influencing fish farming's success or failure is water quality. Probiotics are essential for maintaining water quality and enhancing fish health[93]. Probiotics like *Bacillus licheniformis* and *B. subtilis* enhanced the quality of aquaculture by regulating the levels of ammonia within acceptable limits[99]. Commercial probiotics that contain *Saccharomyces cerevisiae, Bacillus sp., Nitrobacter sp.,* and *Nitrosomonas sp.* have been demonstrated to enhance *Litopenaeus vannamei* by significantly lowering concentrations of nitrogen and phosphate in water and boosting beneficial bacterial microbiota[100].

Aquaculture antibodies and inorganic nitrogen compounds management

The concentration of Ammonia is naturally discharged into water by fish health and water quality, and is one of the most prevalent problems in fish farms. It indicates stress, destroys the gills, makes breathing harder, and increases the fish's susceptibility to illness[101]. According to the findings, ammonia levels in the water may be lowered by using specific probiotic strains, like *Bacillus toyonensis, Geobacillus strearothermophilus, Bacillus spp., Pediococcus spp., Enterococcus spp., Rhodopseudomonas spp., Lactobacillus spp.,* and *Saccharomyces spp*[102,103,104].

In *Mozambique tilapia (Oreochromis mossambicus)* ponds, the use of *Pseudomonas stutzeri, Bacillus cereus,* and *Bacillus amyloliquefaciens* also a probiotic product mixture, increases nitrifying bacteria like *Nitrosomonas* and *Nitrobacter*, improving water quality[105,106]. Use of *Nitrobacter* and *Nitrosomonas* in recirculating systems demonstrated the best control over ammonia, nitrite, and nitrate levels, enhancing the quality of water and lowering fish hazards[107].

Additionally, microbes play a crucial role in the nitrogen cycle, which keeps aquatic environments in balance. In aquariums, they create NH4-N and N, and they convert to nitrite and nitrate by denitrifying bacteria[108]. Numerous probiotic microbes were identified as proven to improve water quality by supporting microbes that are beneficial for nitrification, they convert ammonia to nitrite, and nitrate to nitrogen gas. Probiotics also aid in the breakdown of organic debris, which lowers the pond's nitrogen compounds. They thus represent the most effective approach to lowering the amounts of nitrite and ammonia in fish tank water[108]. However, the success rate of water-based probiotics depends substantially on variables in the environment, which include salinity, pH, and temperature, which are frequently disregarded in controlled studies, causing inconsistent field results. This emphasizes significant field research that investigates the advantages of probiotics in environments such as agriculture.

Probiotic-mediated reduction of BOD and COD in aquaculture systems

By improving the quality of water and decomposing organic waste, probiotics may decrease chemical and biochemical oxygen demand, in fish tanks. Beneficial microbes that decompose organic debris are introduced to do this. It is primarily dependent on heterotrophic microorganisms. Probiotics, beneficial microbes, it is efficient in breaking down leftover and extracting organic waste from excrement of fish, which decreases organic deposition in the water and rapidly reduces BOD and COD levels[109]. Through the conversion of organic matter into carbon dioxide, probiotics are being demonstrated reduce the accumulation of carbon and, during the growth period, dissolved carbon, ultimately improving water quality. This is through minimizing oxygen deficiency induced by infectious or opportunistic microorganisms[110,111]. Additionally, some strains of probiotics encourage the growth of phytoplankton, which aids in photosynthesis[112], the natural process that produces oxygen. Probiotics support maintains liquified oxygen levels and avoid rapid reductions that may kill the aquatic organisms by stabilizing microscopic populations and preventing the formation of ammonia, nitrite, and other harmful chemicals[113]. In general, utilizing them encourages an aquatic environment that may be more sustainable and supplies species of cultivation with better oxygen availability. In the transformation of large amounts of organic materials into masses of bacteria, Gram-positive *Bacillus species* are capable of changing biological substance into carbon dioxide[114].

The fragrance of the water is affected by organic materials, and if percentage is higher than what is allowed, it creates fish-harming compounds like ammonia and H_2S and raises the pH level, which have an impact on the fish's health[115]. A key constraint is the absence of a consistent approach for quantifying the enduring consequences of probiotics on BOD and COD in various kinds of production systems, making it impossible to design a consistent guideline for use[116].

Effects of probiotic activity on hydrogen sulfide levels in aquaculture

Aquatic animals are poisoned by hydrogen sulfide (H_2S), which is created when sulfur-reducing bacteria accidentally break down organic molecules in anaerobic environments. In fish like *Atlantic salmon* and Zebrafish, it may induce quick mortality or sublethal stress by impairing cytochrome oxidase in mitochondria, which reduces oxygen uptake and inhibits cellular respiration[117,118]. Additionally, they inhibit the buildup of silt that normally produces hydrogen sulfide by speeding up the degradation of fish feed and leftover. Through promoting the development of beneficial bacteria and maintaining a healthy microbial biological system[119,120].

International legal frameworks concerning probiotics in aquaculture

Probiotics in fisheries have been subject to various regulations across every continent. Specific rules are enacted by the European Union (EU), which specify that probiotics be non-toxic, noninvasive, and carefully evaluated for protection, traceability and effectiveness. These necessities involve strain quantity identification and surveillance for horizontal transfer of genes and antibiotic resistance. In order to find unanticipated adverse consequences, the EU further requires post-market observation[121].

Probiotics are controlled by the FDA of the United States of America (USA) as Generally Recognized as Safe substances or additives to feed, with an emphasis on protection for humans, the ecosystem, and the species being targeted. The Food and Drug Administration have greater flexibility than the EU as it involves strain-level recognition and biosafety examination[28,122]. Despite being the most significant aquaculture supplier in Asia, and particularly China, regulation is laxer. Probiotics and symbiotics are frequently utilized but inadequate quality control, an absence of strain identification, and problems with biosafety emphasize the need for strengthened regulatory frameworks[123,124,125].

Probiotic guidelines in African aquaculture are now being developed. Guidelines for the consumption of probiotics have started from being established in specific countries, like Tanzania and Nigeria, with emphasis on environmental effect, safety, and efficacy. Comprehensive standards along with capacity building are required, but enforcement and consistent mechanisms remain inadequate[126].

Innovative methods for monitoring and administration of probiotics in aquaculture

Innovative distribution mechanisms are currently investigated to increase probiotic persistence and potency in aquaculture. Probiotics might be effectively introduced to the early stages of larval development through bioencapsulation in fresh food, including *Artemia* and *rotifers*, which enhances gut colonization and survival[127,128,129]. Comparably, probiotic or symbiotic-loaded functional nutrition are being created to offer calibrated dosages while conserving viability of bacteria during feeding and storage. As bioremediators, probiotics are directly released to the water environment to enhance water quality, increase dissolved oxygen, and inhibit advantageous infections by exclusion from competition[130,131,132] (Fig. 2).

Next-generation sequencing and metagenomics have transformed probiotic studies through the provision of high-resolution microbial ecosystem screening and monitoring[133,134]. Selection researchers may more easily assess any biosafety concern, such as horizontal gene transfer, and track probiotic colonization and persistence in aquaculture systems and the host gut attributable to these resources. NGS enhances the scientific foundation for probiotic application for offering community-wide insights[135].

The focus have shifted from traditional probiotics to postbiotics, synbiotics and genetically modified probiotics[136,137]. Probiotics and prebiotics are combined in synbiotics to improve colonization and activity, which enhances host health in a synergistic way[138,139]. An additional degree of biosafety is provided by postbiotics; they have functional effects without requiring live things and are composed of metabolites or inert microbial cells[140,141,142]. Genetically modified probiotics, which are intended to carry antimicrobial peptides, vaccinations, or enzymes that enhance food absorption, are becoming more feasible due to advancements in biotechnology. These developments show promise for future generation of aquatic cultivation techniques, even if they are currently mostly experimental[143,144]. Although many of these inventions remain in their early phases of development, their long-term ecological effects and economic viability for extensive commercial use remain to be fully assessed.

Conclusion and instructions for the future

It has been observed that adding probiotics to water and feed is very advantageous. Enhance immunity, higher quality of water, reduce requirement for water exchange, increased growth and survival of fish, and these microbes enable a generally healthier raising environment. In addition, Probiotics maximise feed utilization, improve disease resistance, and support reproductive health. Therefore, their use promotes the objectives of environmentally conscious and sustainable aquaculture practices while simultaneously enhancing the productivity and welfare of cultured fish. The observed variety in probiotic efficiency may be due to changes in the experimental and sampling procedures, the type of fish used, and the quantities of probiotic strains used.

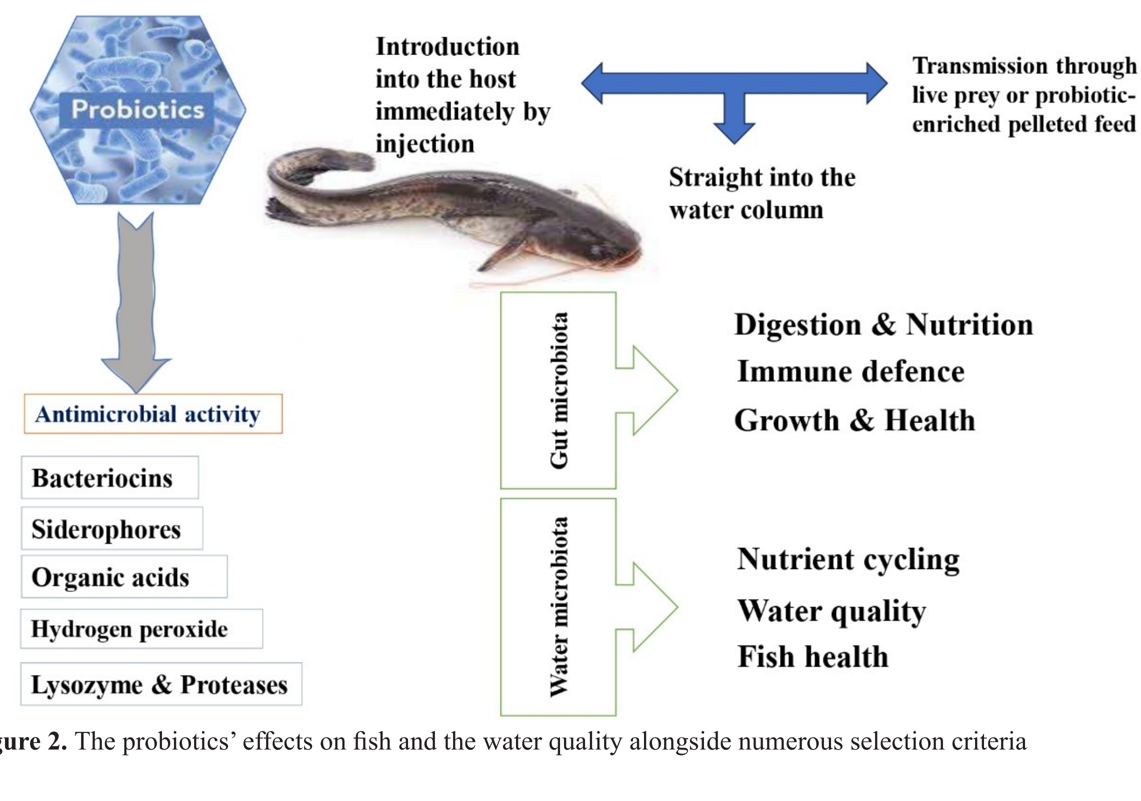

Figure 2. The probiotics' effects on fish and the water quality alongside numerous selection criteria

To investigate crucial commercial and scientific issues, future aquaculture probiotic research focus on the following areas in addition to simple efficacy studies: standardizing procedures for selecting and estimating probiotic dosages for a various kind of shellfish species under a range of environmental conditions (like varying salinities, temperatures, and pH) is required for producing standardized, predictable results for aquaculture producers. Developing more targeted and effective probiotic strains by using technologies like metagenomics, transcriptomics, and proteomics to gain a deeper comprehension of the precise processes by which probiotics interact with the host immune system and gut microbiota. Future research should concentrate on creating probiotic strains that are verified to be free of mobile ARGs and creating precise regulatory frameworks for use. A comprehensive examination of the dangers of ARGs horizontal gene transfer from probiotics to pathogens. Investigating and refining novel formulations that would offer improved stability, a longer shelf life, and increased safety, such as synbiotics and postbiotics.

Author contributions

Vishal Rajput: Conceptualization, validation, writing-original draft preparation; Divya Arya: writing-original draft preparation; Sanjay Gupta: Writing-review and editing.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

The conducted research was not related to either human or animal use.

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