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Original Article

Recent advances and challenges in the industrial-scale production of probiotics in aquaculture

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Abstract: Probiotics have emerged as a transformative solution for improving health, growth, and disease resistance in aquaculture, offering a sustainable alternative to conventional practices. This review focuses on updates and advancements in the industrial-scale production of probiotics for aquaculture, emphasizing their significance in fostering eco-friendly aquaculture systems. The development of probiotics tailored for aquaculture has seen substantial progress, with species like *Bacillus*, *Lactobacillus*, and *Saccharomyces* dominating the market due to their proven benefits. Emerging technologies are revolutionizing industrial production, including large-scale fermentation techniques designed to enhance yield and maintain viability, alongside innovative microencapsulation methods that improve the stability and shelf-life of probiotic formulations. Advances in quality control and standardization are also enabling the production of high-quality, consistent products, meeting both industry demands and regulatory standards. However, industrial production is not without its challenges. Technical issues, such as optimizing strains for mass production and ensuring their functionality under diverse aquaculture conditions, remain critical hurdles. Economic and regulatory barriers, including high production costs and stringent compliance requirements, further complicate large-scale implementation. Despite these challenges, the sector holds immense potential, with emerging opportunities in strain optimization and integrative approaches using biofloc systems and advanced feed formulations. This review highlights the ongoing evolution of industrial probiotic production and underscores the need for multidisciplinary collaboration to address existing barriers. By leveraging technological innovations and fostering industry-academia partnerships, aquaculture can achieve more sustainable and efficient practices, paving the way for the broader application of probiotics on an industrial scale.

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Introduction

The global population is projected to reach 9.7 billion by 2050 and 10.4 billion by 2100 (Legg, 2024), which will significantly strain food security, especially as climate change exacerbates these challenges (Maulu et al., 2021). To address these growing concerns, aquaculture has emerged as a sustainable source of high-quality protein, vitamins, and minerals (Bohnes et al., 2022). This sector presents a potential solution to meet the rising demand for food in a rapidly growing world. However, the major challenge lies in whether aquaculture can scale quickly and sustainably enough to keep pace with this demand, particularly given the pressures posed by climate change. As global food production already faces immense challenges, climate change threatens to further undermine the quality and quantity of food available (Myers et al., 2017). The ability of aquaculture to adapt and expand under these circumstances is crucial in securing a sustainable food future for an increasingly populated world.

The 2024 State of World Fisheries and Aquaculture (SOFIA) reported that global fisheries and aquaculture production reached 223.2 million tons in 2022, reflecting a 4.4% increase from 2020, noting that the production includes 185.4 million tons of aquatic

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animals (FAO, 2024). Within this global context, the South Asian region, which includes Afghanistan, Bangladesh, Bhutan, India, the Maldives, Nepal, Pakistan, and Sri Lanka, plays a significant role, contributing 23.83 million tons of fish. This represents 12.14% of the world's total fish production and 14.89% of global aquaculture production from animals (Giri, 2024). In Southeast Asia, the Philippines, an archipelagic country consisting of 7,641 islands divided into three main groups, Luzon, Visayas, and Mindanao, contributes notably to regional aquaculture. Luzon and Mindanao are the country's two largest islands (Tahiluddin and Terzi, 2021). In 2022, aquaculture production in the Philippines reached 2.35 million metric tons, valued at approximately PhP 124.00 billion, according to the Bureau of Fisheries and Aquatic Resources (BFAR), which is responsible for managing and enhancing the country's aquatic resources, playing a key role in development and sustainability (BFAR, 2022).

One of the biggest challenges in the growth of aquaculture has been dealing with outbreaks of pathogens (Mzula et al., 2021). The most common methods for fighting infections have traditionally been the use of chemical agents and antibiotics (Torres-Maravilla et al., 2024). In recent years, however, the use of antibiotics has come under scrutiny and has been restricted in many countries (Luthman et al., 2024), because of their bio-accumulative effects and the rise in antibiotic-resistant bacteria, which have harmful consequences for both human and animal health (Santos and Ramos, 2018; Pepi and Focardi, 2021). Alternatives to the excessive use of antibiotics in aquaculture include vaccination and the use of probiotic strains (Bondad-Reantaso et al., 2023). Merrifield et al. (2010) expanded on this definition specifically for aquaculture, describing probiotics as organisms—alive, dead, or components of microbial cells—that, when added through feed or rearing water, enhance the host's health by improving disease resistance, growth performance, feed utilization, and stress response. This review aims to provide an updated overview of the role of probiotics in aquaculture, emphasizing recent advancements in industrial-scale production.

Probiotics developed for aquaculture

Commonly used probiotic strains: The use of probiotics in aquaculture is underpinned by their ability to improve the health and productivity of aquatic organisms through diverse mechanisms. Among the bacterial genera commonly used as probiotics, *lactic acid bacteria* (*LAB*), *Bacillus*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, and *Streptomyces* have been identified as particularly effective (Ringø, 2020). In addition to bacterial strains, eukaryotic microorganisms such as microalgae like *Tetraselmis* and yeasts from genera *Debaryomyces*, *Phaffia*, and *Saccharomyces* have demonstrated significant probiotic efficacy (Ringø et al., 2020). These diverse microorganisms provide valuable tools for enhancing aquaculture practices, and strains such as *Aeromonas media* (e.g., strain A199), *Bacillus subtilis*, *Lactobacillus helveticus*, *Enterococcus faecium*, and *Carnobacterium inhibens* are currently recognized for their significant effectiveness in aquaculture systems (Fachri et al., 2024).

The functional traits of probiotics are critical to their success in aquaculture. These include the promotion of growth through enhanced digestive enzyme activity and improved nutrient absorption (Mohammadi et al., 2022). Additionally, probiotics modulate the immune system, increasing the resistance of aquatic organisms to infections and reducing mortality rates. For example, dietary supplementation with *Bacillus* spp. in red hybrid tilapia has been shown to reduce cumulative mortality and viral load (Waiyamitra et al., 2020). Another promising approach involves competitive exclusion, where beneficial microorganisms inhibit the colonization and growth of pathogens by occupying ecological niches within the host, as demonstrated by the study of Nile tilapia microbiota-derived probiotics (Melo-Bolívar et al., 2022). These benefits collectively reduce the reliance on antibiotics, thereby supporting sustainable aquaculture practices

(Hoseinifar et al., 2018; Torres-Maravilla et al., 2024). Probiotic supplementation has also shown potential for mitigating stress in aquaculture environments. Under heat stress, dietary *Bacillus* probiotics significantly improved growth performance, enhanced antioxidant enzyme activity, and modulated immune responses while reducing oxidative stress markers and biochemical indicators of stress in Nile tilapia (Elbahnaswy et al., 2024).

In addition to laboratory-based probiotics, Table 1 enumerates a variety of commercially available probiotics and paraprobiotics that have been experimentally validated and are widely used in aquaculture practices. These products have demonstrated effectiveness in improving fish health, growth, and environmental conditions. While both probiotics and paraprobiotics contribute to enhancing aquaculture outcomes, they differ in their functionality. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host, primarily through colonizing the gastrointestinal tract and improving microbial balance (Martínez Cruz et al., 2012). In contrast, paraprobiotics consist of inactivated or heat-killed microorganisms that exert health benefits without requiring colonization, often by stimulating immune responses or delivering bioactive compounds (Li and Tran, 2022).

Several commercially available probiotic products have been developed, each with unique formulations and reported benefits. For instance, Agrimos, produced by Lallemand Animal Nutrition in Denmark, contains *Saccharomyces cerevisiae* and has been shown to enhance animal performance, balance gut microbiota, and preserve gut integrity (Mohamed et al., 2017). Similarly, Biomin's AquaStar, a blend of *B. subtilis, E. faecium, Lactobacillus reuteri,* and *Pediococcus acidilactici*, improves larval survival, boosts immune responses, and enhances growth performance (Azimirad et al., 2016). Lallemand Animal Nutrition offers another product, Bactocell, containing *P. acidilactici*, which improves feed efficiency, enhances immune responses, and promotes better digestibility (Hoseinifar et al., 2015). On the other hand, Biogut, a product by Varsha Group in India, comprises multiple strains, including *Lactobacillus sporogenes, L. acidophilus, B. subtilis, B. licheniformis,* and *S. cerevisiae*. This formulation is effective in improving survival rates, disease resistance, and immunity against white muscle disease (Pavadi et al., 2018). Bioplus, produced by Chr. Hansen, in Denmark, combines *B. subtilis* and *B. licheniformis* to enhance growth performance, feed utilization, and disease resistance (Ridha and Azad, 2012).

Keeton Industries' EcoPro-A, a U.S.-based product, contains *B. subtilis, B. licheniformis, B. megaterium,* and *P. acidilactici*, offering benefits such as improved growth, enhanced immune function, and better water quality (Nayak, 2010). Similarly, Efinol from Bentoli Agrinutrition in the United States is formulated with *B. subtilis, P. acidilactici,* and *S. cerevisiae*, leading to enhanced growth, improved feed efficiency, and greater stress tolerance (Hauville et al., 2016). Another notable product, Epicin, developed by Epicore Bionetworks Inc. in the United States, comprises *Bacillus* spp., *Pediococcus* spp., and *Enterococcus* spp., improving water quality, growth, and disease resistance (Balcázar et al., 2006). In Brazil, ICC's Hilyses® includes *S. cerevisiae* and has demonstrated benefits such as enhanced immune response, improved digestive health, better growth performance, reduced stress, and increased disease resistance while supporting gut microbiota balance (Muñoz-Atienza et al., 2015). Japan's Morinaga Milk Industry offers LAC-Shield™**,** featuring heat-killed *Lacticaseibacillus paracasei* MCC1849, which modulates the fish immune system and aids in combating bacterial infections (Murata et al., 2018; Lensch et al., 2024). Similarly, Lacteol® diarrhEase™, a heat-killed *L. acidophilus* product by Lacteol in France, has been reported to improve growth performance, antioxidant capacity, and immune system strength in various fish species (Liévin-Le Moal, 2016; Ringø et al., 2018; Barui et al., 2024).

Additional products include LevuCell, another Lallemand Animal Nutrition product containing

Table 1. Commercially available probiotics used in aquaculture.

Product Name	Company (Country)	Composition
Agrimos	Lallemand Animal Nutrition (Denmark)	*S. cerevisiae*
AlCare	Alpharma Inc (USA)	*B. licheniformis*, *B. subtilis*
AquaStar	Biomin (Austria)	*B. subtilis*, *E. faecium*, *L. reuteri*, *P. acidilactici*
Bactocell	Lallemand Animal Nutrition (France)	*P. acidilactici*
Biogut	Varsha Group (India)	Mix including *Lactobacillus* spp., *B. subtilis*, *S. cerevisiae*
Bioplus	Chr. Hansen (Denmark)	*B. subtilis*, *B. licheniformis*
EcoPro-Aqua	Keeton Industries (USA)	*B. subtilis*, *B. licheniformis*, *B. megaterium*, *P. acidilactici*
Ecobiol	Evonik (Germany)	*Bacillus amyloliquefaciens* CECT 5940
Efinol	Bentoli Agrinutrition (USA)	*B. subtilis*, *P. acidilactici*, *S. cerevisiae*
Epicin	Epicore BioNetworks Inc (USA)	*Bacillus* spp., *Pediococcus* spp., *Enterococcus* spp.
Hilyses®	ICC (Brazil)	*S. cerevisiae*
LAC-Shield™	Morinaga Milk Industry (Japan)	Heat-inactivated *Lactiplantibacillus paracasei* MCC1849
Lacteol® diarrEase™	Lacteol (France)	Heat-killed *L. acidophilus* LB
Levucell	Lallemand Animal Nutrition (France)	*Saccharomyces cerevisiae boulardii*
Protexin Aquatech	Probiotics International Ltd (UK)	Multi-strain mix including *B. subtilis*, *L. rhamnosus*, *E. faecium*
Sanolife PRO-F	INVE Aquaculture (Belgium)	*B. subtilis*, *B. licheniformis*, *B. pumilus*
Staimune®	Ganeden (USA)	Heat-treated *Hendrickxia coagulans* (*Bacillus coagulans*)
Toyocerin	Rubinum (Spain)	*Bacillus cereus var. toyoi*

Saccharomyces cerevisiae boulardii, which enhances growth, immune response, and stress tolerance (Perdichizzi et al., 2023). Protexin Aquatech, from Probiotics International Ltd in the United Kingdom, is a multi-strain probiotic including *B. subtilis, Lactobacillus rhamnosus,* and *E. faecium*, designed to improve growth, disease resistance, and water quality (Mohapatra et al., 2012). Sanolife PRO-F, developed by INVE Aquaculture in Belgium, combines *B. subtilis, B. licheniformis,* and *B. pumilus*, offering benefits such as improved feed conversion, enhanced survival rates, and better water quality (van Hai and Fotedar, 2010). Finally, Ganeden's Staimune™, based in the United States, utilizes heat-killed *Hendrickia coagulans* (previously *Bacillus coagulans*), which has been shown to enhance growth, immune response, and disease resistance in fish (Endres et al., 2009; James et al., 2021; Omar et al., 2024). Toyocerin, produced by Rubinum in Spain, contains *Bacillus cereus* var. *toyoi* and is effective in encouraging growth, improving specimen homogeneity, and enhancing intestinal mucosa (Abdulmawjood et al., 2019).

Methods of probiotic administration

Feeding additives**:** Being one of the most common approaches, probiotics are either added to the feed in liquid or dry form or incorporated into commercial aquafeed formulations (Sumon et al., 2022). They can also be manually mixed with feed during production (Emam et al., 2022). The appropriate dosage of probiotics must be determined based on the specific needs of the aquatic species to ensure optimal benefits without adverse effects (Kechagia et al., 2013). For example, in keureling fish (*Tor tambra*) fry, the optimal probiotic dosage was identified as 10 ml per kg of feed (Muchlisin et al., 2017). Another example is applying probiotics containing *B. subtilis* at dosages of 5 to 7.5 mg/L, which significantly enhances the survival, development, and crablet production of *Scylla tranquebarica* larvae by reducing *Vibrio* spp., ammonia, and total organic matter in the rearing environment (Gunarto et al., 2024). It is important to note that the recommended dosages may vary between species (Jahangiri and Esteban, 2018).

Bath treatments: This process involves placing aquatic organisms in a solution containing probiotics, allowing direct contact with the beneficial microorganisms (Yaslikan et al., 2023). This method is particularly effective during the early stages of larval development. Treating zebrafish larvae with a probiotic solution containing live lactic acid bacteria enhances early growth, development, digestive function, and survival, aligning with the principles of probiotics bath treatment (Padeniya et al., 2022). Additionally, *L. acidophilus* treatments improved growth performance, digestive enzyme activity, and disease resistance in juvenile grass carp, with effects persisting for at least four weeks post-treatment (Yaslikan et al., 2023).

Probiotics in Biofloc systems: Biofloc systems represent an innovative aquaculture approach that leverages microbial communities to convert waste into protein-rich food for aquatic organisms (Crab et al., 2012). Enzymes and other substances produced within biofloc systems help break down feed components and increase nutrient bioavailability (Kumar et al., 2021). Studies have demonstrated that biofloc systems improve growth performance, feed conversion ratios, and biomass in species like Nile tilapia (Mohammadi et al., 2020). Recently, research by He et al. (2023) highlighted the benefits of adding *B. subtilis* to biofloc systems, which enhanced water quality, growth, and immune enzyme activity in *Litopenaeus vannamei.* Biofloc systems utilize nutrient-rich microbial communities as a mode of probiotic administration, supporting enhanced growth and feed efficiency in aquatic animals (Wei et al., 2024).

Recent advancements in strain development: Bacterial strains employed in aquaculture differ from those utilized for human consumption, but they provide similar health benefits and continue to be extensively studied for their effectiveness in aquaculture systems. For example, Muñoz-Atienza et al. (2014) identified *Leuconostoc mesenteroides* subsp. *cremoris* SMM69 and *Weissella cibaria* P71 as promising probiotic candidates for turbot farming. These strains demonstrated robust antimicrobial activity against *Tenacibaculum maritimum* and *Vibrio splendidus,* exhibited excellent probiotic properties, and effectively inhibited pathogen adhesion to mucus. Furthermore, they were deemed safe for turbot larvae and juveniles, stimulating immunity-related gene expression, particularly in mucosal tissues, thereby reinforcing non-specific immunity.

Recombinant probiotics: Advancements in biotechnology have paved the way for next-generation recombinant probiotics, which are engineered to exert targeted effects on the host, such as boosting immune responses or modulating microbiota composition and metabolic activity (Fig. 1). For instance, Santos et al. (2020) developed a genetically engineered *B. subtilis* strain capable of producing and secreting fungal phytase to counteract the anti-nutritional effects of phytate in vegetable-based fish diets. Feeding fish with this transgenic probiotic improved growth performance, nutrient absorption, and bone metabolism, as demonstrated by the upregulation of genes associated with peptide transport, growth, and metabolism. Similarly, Nakharuthai et al. (2023) identified and engineered *Bacillus* spp. isolates from Nile tilapia intestines, particularly isolate B29 (closely related to *B. subtilis*), to deliver CC chemokine proteins. The recombinant probiotics significantly enhanced immune responses in fish compared to wild-type probiotics, demonstrating the superior immunostimulatory capabilities of genetically modified strains.

Moreover, recombinant probiotics have shown potential beyond basic nutrient enhancement. For example, Bandari et al. (2024) demonstrated the effectiveness of bioengineered *Lactococcus lactis* expressing phytase enzymes in improving phosphorus bioavailability and mitigating the antinutritional effects of phytate in plant-based diets for livestock, poultry, and aquaculture species. This engineered strain effectively produced and delivered phytase, enhancing feed utilization efficiency and supporting growth performance. Additionally, *L. lactis* has been explored as a vehicle for delivering therapeutic molecules, as shown in Muñoz et al. (2021), which engineered *L. lactis* to express type I interferon, which stimulated antiviral immune responses in Atlantic salmon. Oral administration of this recombinant

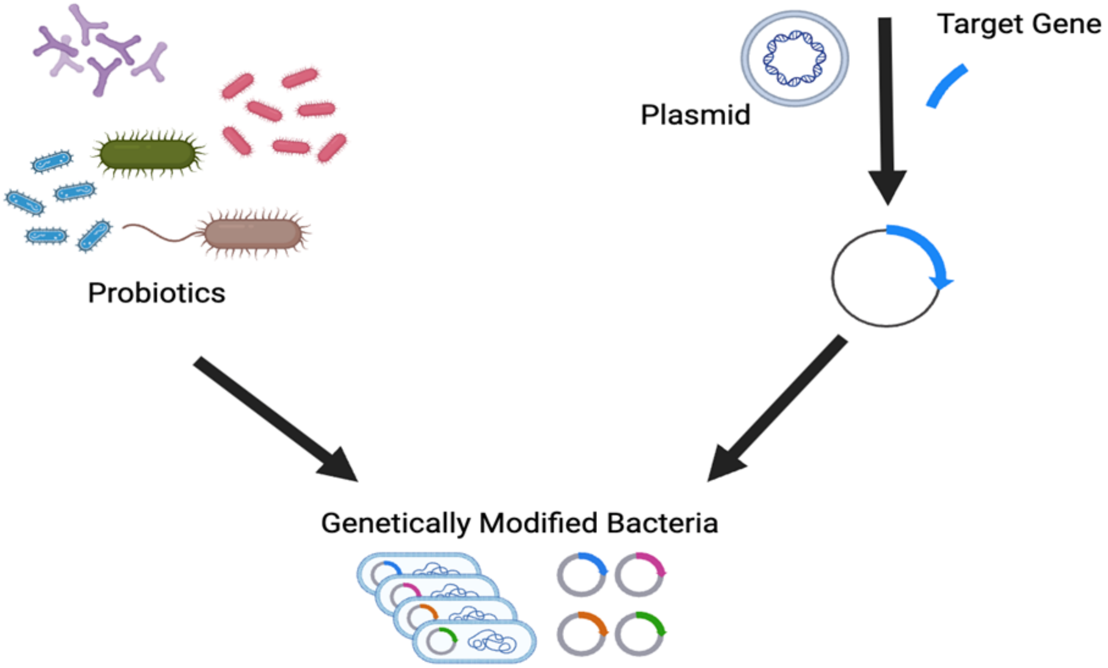

Figure 1. The process of producing recombinant probionts. Adapted from Torres-Maravilla et al. (2024). Figure created with BioRender.com.

probiotic increased the expression of antiviral markers such as Mx and PKR, reduced infectious pancreatic necrosis virus (IPNV) viral load, and showed systemic effects in immune-related organs like the spleen and head kidney. The findings from these studies highlight the transformative potential of recombinant probiotics in aquaculture. Engineered strains such as *B. subtilis* and *L. lactis* offer innovative solutions to address challenges in nutrient bioavailability, disease mitigation, and immune modulation. These advancements demonstrate significant progress in leveraging bioengineering to improve aquaculture productivity and sustainability.

Emerging technologies for industrial probiotic production

Use of bioreactors and recirculating aquaculture system (RAS): A bioreactor is a vessel or system designed to support and optimize biological processes, such as cell growth, enzymatic reactions, or microbial fermentation, to produce desired products or perform beneficial biotransformation (Jaibiba et al., 2020). It requires careful consideration of genetic expressions, stoichiometry, reaction kinetics, and physical parameters like pH, temperature, and oxygen transfer to achieve efficient and scalable performance (Wang and Zhong, 2007). The integration of bioreactors in aquaculture has revolutionized the production of probiotics, particularly when used alongside RAS technology. RAS is recognized as a sustainable approach to aquaculture due to its environmental compatibility, scalability, and ability to produce higher yields in limited spaces while reducing susceptibility to disease outbreaks (Boaventura et al., 2018; Qi et al., 2020). By recycling 90-99% of water for reuse, RAS systems significantly conserve resources, making them particularly advantageous in regions where water and energy are scarce compared to traditional aquaculture methods (Zhu et al., 2016; Gichana et al., 2018). RAS provides precise control over cultural and environmental conditions, which is especially beneficial for fish purging before reaching the market. Controlled systems enhance fish quality and minimize the presence of harmful metabolites (Azaria and van Rijn, 2018). A critical feature of RAS is the oxidation of nitrogenous waste, such as ammonia, which is essential for maintaining water quality and ensuring a healthy environment for aquatic organisms (Kinyage et al., 2019).

Bioreactors play a pivotal role in supporting these systems by enabling the efficient treatment of wastewater generated by RAS. Biological processes in bioreactors are considered eco-friendly and cost-effective solutions, demonstrating high treatment performance in removing nitrogenous and organic waste (Liu et al., 2019; Rahimi et al., 2020; Waqas et al., 2020). Optimizing bioreactor conditions, such as the rate of substrate addition, is crucial for enhancing the metabolic activities of probiotics. Substrate concentration in the growth medium directly influences cell growth rate, oxygen consumption, and the production rates of desired products and by-products, all of which are essential factors for industrial probiotic production (Mears et al., 2017). The use of bioreactors in large-scale fermentation not only enhances the efficiency of probiotic production but also aligns with the sustainability goals of aquaculture. By ensuring precise control of fermentation parameters and maximizing resource efficiency, bioreactors are paving the way for the future of aquaculture-specific probiotics.

Batch fermentation process: In a batch fermentation process, a specific volume of medium is introduced to a microbial culture, where microorganisms utilize the available nutrients for growth while producing by-products. As the process continues, nutrient depletion and by-product accumulation occur, eventually slowing microbial growth and transitioning the culture into the stationary phase (Bolmanis et al., 2023). This method is simple to operate, as all carbon sources and media components are added at the beginning of the process, and fermentation proceeds until the carbon source is exhausted (Abdel-Rahman et al., 2013). However, despite its simplicity, batch fermentation has notable limitations, such as lengthy downtime during batch turnaround, fluctuating substrate concentrations, and limited control over microbial growth rates and product formation, which collectively reduce its efficiency (Mears et al., 2017). Recent advancements aim to address these limitations by exploring alternative fermentation strategies and media formulations. A study by Śliżewska and Chlebicz-Wójcik (2020) investigated the growth kinetics of probiotic *Lactobacillus* strains in a novel semi-solid fermentation (SSF) medium. This research compared the performance of the SSF medium to the conventional de Man, Rogosa and Sharpe (MRS) medium. While the SSF medium extended the lag phase of *Lactobacillus* strains (ranging from 1.98 to 5.64 hours), it supported a maximum growth rate that was twice as high as that observed in the MRS medium. These findings underscore the SSF medium's potential as a viable and efficient alternative for large-scale cultivation of *Lactobacillus* spp.

Fed-batch fermentation process: Fed-batch fermentation is a widely adopted bioprocessing method, especially for achieving high cell densities and product concentrations (Yang and Zhang, 2018). Unlike batch fermentation, where all nutrients are added at the beginning, or continuous fermentation, where nutrients are supplied and removed simultaneously to maintain a steady state, fed-batch fermentation involves the controlled addition of nutrients throughout the process (Doran, 2013). This method allows for substantial biomass accumulation while preventing the depletion of essential nutrients and minimizing the accumulation of inhibitory by-products (Lim and Shin, 2011). By carefully regulating substrate addition, overfeeding is avoided, leading to improved product and biomass yields per unit of substrate provided (Lee et al., 2003).

Fed-batch fermentation is particularly advantageous when the desired product is directly associated with microbial growth, offering more flexibility and higher yields compared to batch fermentation, which has limited control over growth rates and product formation (Rajpurohit and Eiteman, 2022). The reliability and high product yields per cycle have made fed-batch the standard for many industrial bioprocesses, surpassing both batch and continuous methods in certain applications (Lindskog, 2018). For example, Beitel et al. (2020) demonstrated the effectiveness of fed-batch fermentation for enhancing lactic acid production using *Lactobacillus delbrueckii*. By employing low-cost substrates such as molasses and corn-steep liquor, their study achieved a remarkable lactic acid concentration of 162 g/L within 48 hours under fed-batch conditions.

Continuous fermentation process: Continuous

Table 2. Industrial fermentation processes and their application in aquaculture probiotics.

Method	Description	Advantages	Limitations	Application
Batch Fermentation	A closed system where nutrients are supplied at the beginning, and fermentation proceeds until nutrients are exhausted.	Simple setup; suitable for small-scale production; effective for short-term microbial cultivation.	Inefficient for large-scale production; downtime between batches; accumulation of inhibitory by-products.	Used for producing probiotics for research and small aquaculture facilities (Bolmanis et al., 2023).
Fed-batch Fermentation	Controlled addition of nutrients during the process to avoid depletion and maintain high cell densities.	Achieves high cell densities and product concentrations; flexible control of nutrient supply.	Requires careful monitoring and regulation; higher setup complexity compared to batch fermentation.	Production of high-quality probiotics like *Lactobacillus* for aquaculture feed additives (Beitel et al., 2020).
Continuous Fermentation	Continuous input of fresh medium and removal of spent medium to maintain steady-state conditions.	Stable and consistent production; reduced operational costs over time; higher efficiency.	High risk of contamination; genetic drift; complex process control; limited industrial use in aquaculture.	Production of probiotics with enhanced metabolic activity using immobilized cell systems (Desmond et al., 2004).

fermentation involves the steady addition of fresh cultivation medium into the bioreactor while simultaneously removing spent medium and cells (Kuenen, 2019). This process ensures a constant culture volume, allowing for the replenishment of nutrients and removal of toxic metabolites, thereby maintaining a stable medium level in the bioreactor (Bolmanis et al., 2023). This method is particularly valued for its ability to achieve lower operational costs and stable production rates (Chen et al., 2021). However, while continuous fermentation offers significant advantages as a research tool due to its capacity for maintaining sustained microbial growth, it faces challenges in industrial applications. Issues such as a high risk of contamination, genetic drift within cultures, and the complexities of maintaining precise process control have limited its large-scale use, making it less prevalent than batch and fed-batch systems (Xie, 2022). Despite these challenges, innovations like continuous immobilized cell (IC) cultures have demonstrated promising potential (Genisheva et al., 2014). Desmond et al. (2004) highlighted that IC systems can produce probiotics with enhanced viability and metabolic activity. Unlike conventional batch fermentation, where cells often experience nutrient depletion, IC technology allows cells to remain in the exponential or early stationary growth phase, resulting in improved tolerance to environmental stresses. This approach eliminates the need for preconditioning treatments that, while increasing survival, can compromise cell activity and yield. Table 2 provides a summary of the description, advantages, limitations, and application of batch, fed-batch, and continuous fermentation processes.

Microencapsulation, quality control, and standardization: A pivotal technology in safeguarding probiotics during storage and application, addressing their inherent sensitivity to environmental stressors such as heat, oxygen, moisture, and light (Sun et al., 2023). By forming a protective barrier, encapsulation ensures the viability and effectiveness of probiotics even after prolonged storage (Rodrigues et al., 2020). This is particularly critical for maintaining their stability until they reach their target location in the gastrointestinal (GI) tract, where they can exert their beneficial effects (Markowiak and Śliżewska, 2017; Maftei et al., 2024; Yang et al., 2024). Moreover, encapsulation significantly improves the controlled release of probiotics, protecting them from harsh digestive conditions, such as stomach acid and bile salts, thus enhancing their survival and functionality in the gut (Ozturk et al., 2021; Agriopoulou et al., 2023; Latif et al., 2023).

Advances in quality control and standardization have further supported the development and application of encapsulation technologies (Moran et al., 2014). Rigorous testing protocols, optimized fermentation processes, and regulatory frameworks ensure that encapsulated probiotics meet high standards for stability and efficacy (Araujo et al., 2024; Wang et al., 2025). For example, encapsulating strains like *S. cerevisiae* in alginate beads has proven effective in preserving their viability during high-temperature feed processing, ensuring consistent functionality in aquaculture (Bevilacqua et al., 2020). Regulatory requirements, such as those outlined by Food and Agriculture Organization (FAO), World Health Organization (WHO) and regional bodies like the European Food Safety Authority (EFSA), emphasize safety parameters, including the absence of transferable antibiotic resistance genes and pathogenicity, which guide encapsulation practices (Rahayu et al., 2024).

Whole Genome Sequencing (WGS) has emerged as a cornerstone of quality control, enabling precise strain characterization (Wang et al., 2021; Mustafa, 2024). This ensures the selection of probiotics with beneficial traits like enzyme production, immune modulation, and pathogen resistance, while also confirming the absence of undesirable features such as virulence or antibiotic resistance (Wang et al., 2021; Dhanya Raj et al., 2023). For instance, WGS analysis of the marine-derived *Bacillus amyloliquefaciens* strain BTSS3, isolated from deep-sea shark *Centroscyllium fabricii*, exhibits antimicrobial and probiotic properties, containing genes associated with vitamin production, secondary metabolite biosynthesis, GI tract survival, and intestinal mucosa adhesion (D'Rose and Bhat, 2023).

Probiotic safety in aquatic systems is evaluated using in vivo pathogenicity tests involving fish or other aquatic organisms (Calcagnile et al., 2024). Fish are exposed to potential probiotics through injection, oral administration, or immersion, and their response is monitored for clinical symptoms or mortality (Assefa and Abunna, 2018). For environmental safety, simpler organisms such as *Daphnia magna*, *Chlorella vulgaris*, or *Brachionus calyciflorus* are often used due to ease of cultivation, rapid lifecycle, and cost-efficiency (Faramarzi et al., 2012; Chen et al., 2020; Abdel-Latif et al., 2022; Zhang et al., 2023). However, their biological differences from fish limit the direct applicability of the results, necessitating more complex models, such as those using zebrafish, for an accurate assessment. Zebrafish models are widely used for acute and chronic toxicity evaluations, with endpoints like behavioral changes, growth performance, and histological examinations (Chen et al., 2020). Zebrafish are the most frequently employed vertebrate models for probiotic safety studies due to their ease of maintenance, ethical acceptance, and genetic similarity to higher vertebrates (Xiong et al., 2022). Acute toxicity tests with zebrafish larvae, embryos, or adults determine the lethal concentration (LD50 or LC50) of a probiotic strain, while chronic tests assess long-term exposure effects, including growth performance, survival rates, and behavior (Ali et al., 2011; Pandelides et al., 2024).

Optimized fermentation and encapsulation for targeted delivery: Optimized fermentation processes complement encapsulation techniques by producing higher yields and ensuring the production of contamination-free probiotic cultures. Continuous fermentation systems, coupled with automation and real-time monitoring, maintain optimal conditions for cell growth. For instance, automated pH control and nutrient supplementation during *Bacillus* fermentation have doubled production efficiency compared to traditional methods (Biswas et al., 2023). The integration of encapsulation and optimized fermentation not only preserves probiotic viability but also ensures functional efficacy during storage and application (Agriopoulou et al., 2023). Advances in microencapsulation, such as alginate-based or liposomal techniques, provide targeted delivery mechanisms while enhancing protection against environmental stressors like desiccation and oxidation (Subramani and Ganapathyswamy, 2020; Lai et al., 2024). These innovations are particularly valuable in applications like aquaculture, where probiotics face challenges such as high-temperature feed processing and water quality variations (Terpou et al., 2019).

The convergence of encapsulation technologies and stringent quality control standards has been instrumental in ensuring the safety and efficacy of probiotics (Sun et al., 2023). For instance, regulatory compliance demands have driven innovation in encapsulation processes to meet shelf-life and functional requirements without compromising product safety (Rezagholizade-shirvan et al., 2024). As a result, encapsulation methods like spray fluidized bed drying and coacervation have gained prominence for their ability to provide uniform protection and controlled release of probiotics, addressing both industrial scalability and regulatory benchmarks (Koh et al., 2022; Agriopoulou et al., 2023). Encapsulation technologies, supported by advances in quality control and standardization, have revolutionized the stability, delivery, and functionality of probiotics. As these techniques continue to evolve, they will play an increasingly critical role in meeting the growing demand for effective probiotic products in diverse applications, from human health to aquaculture. Table 3 enumerates the different microencapsulation techniques currently used as part of the quality control and storage of probiotics.

Challenges and solutions in scaling up probiotic production: One of the primary technical challenges in probiotic application is strain stability. Probiotics are sensitive to environmental factors such as temperature and oxygen levels, which can significantly affect their viability during storage and in the digestive systems of aquatic organisms (Wendel, 2022). Elevated temperatures reduce the metabolic activity and viability of probiotics, necessitating stringent storage conditions and tailored formulations for specific aquaculture environments (Aguinaga Bósquez et al., 2022). The colonization of probiotics within aquatic species depends on their reproduction rate surpassing their expulsion rate, making strain selection critical for efficacy (Pandiyan et al., 2013). Cost-effective production of probiotics remains a significant hurdle in scaling their use in aquaculture. Industrial-scale production requires optimized fermentation processes, efficient microbial culture techniques, and rigorous quality control to ensure consistency and efficacy (Kumar et al., 2022). Innovations in bioprocess engineering, including the use of cost-effective substrates and bioreactor design, can help lower production costs while maintaining the functional properties of probiotics (Boodhoo et al., 2022).

The integration of probiotics in aquaculture feed has shown promise in enhancing nutrient bioavailability and digestion. Probiotics improve the enzymatic breakdown of alternative feed sources, which are increasingly derived from plant and animal by-products instead of traditional fish meal and fish oil (Wuertz et al., 2021). However, these alternative sources often lack the unique nutritional properties of traditional marine-based feed, emphasizing the need for advanced bioengineering and enzyme technology to enhance their nutritional profiles (Maksimenko et al., 2024). Downstream processing, including the formulation, encapsulation, and delivery of probiotics, is a critical area requiring innovation (Vivek et al., 2023). The development of microencapsulation technologies can improve the stability of probiotics during storage and their targeted release in aquatic species' digestive systems (Bu et al., 2025). These advancements not only enhance the efficacy of probiotics but also reduce the degradation caused by environmental stressors such as temperature and pH fluctuations (Baral et al., 2021). Probiotics contribute significantly to improving water quality in aquaculture systems by reducing toxic compounds such as ammonia and nitrite (Tabassum et al., 2021). However, environmental factors like climate change and habitat degradation can exacerbate water quality issues, posing challenges to maintaining stable conditions for probiotics to thrive. It is important to ensure water quality management in preventing disease outbreaks and enhancing aquaculture productivity. Innovations in water treatment technologies that incorporate probiotics as bioremediation agents can help address these challenges (Jahangiri and Esteban, 2018; Hassan et al., 2022).

Understanding the specific mechanisms of pathogen inhibition and optimizing the application of probiotics for diverse aquatic species remains an ongoing challenge (Rahayu et al., 2024). Stress tolerance is a critical factor in aquaculture, affecting

Table 3. Microencapsulation techniques for aquaculture probiotics.

Encapsulation Technique	Applications	Challenges
Spray Drying	Probiotic-containing solutions are atomized into fine droplets using a stream of hot air or nitrogen gas, which subsequently dry to form powdered particles (Vivek et al., 2023).	High temperatures may reduce probiotic viability (Paéz et al., 2012).
Freeze-Drying (Lyophilization)	Dehydrating probiotic bacterial cells to enhance their storage stability, particularly for preserving heat-sensitive strains intended for feed or water applications (Gaidhani et al., 2015) (Tyagi et al., 2023).	Expensive; less efficient for large-scale production (Oyinloye and Yoon, 2020; Stratta et al., 2020).
Modified Alginate Beads	Delivering probiotics through feed to protect them in acidic conditions ensures their survival during passage through the stomach, allowing them to reach the intestines where they can exert their beneficial effects. (Masoomi Dezfooli et al., 2022).	Alginate beads alone are less stable in low pH conditions (Oberoi et al., 2021). Modification of alginate beads by an additional coating provides more stability (X. Wang et al., 2022)
Liposomes	Capable of encapsulating hydrophilic, hydrophobic, and amphiphilic substances, making it versatile for a wide range of applications. This property enables the targeted delivery and controlled release of various compounds in different environments (Han et al., 2024).	High costs associated with scaling up and formulations are often relatively dilute aqueous suspensions, leading to extra expenses (Haffner et al., 2016).
Multilayer Emulsions	Colloidal system consisting of at least two immiscible liquids, such as oil and water, where one liquid is dispersed within the other. Emulsions are often employed to encapsulate probiotic cells. The two most common types of simple emulsions are (a) oil-in-water (O/W) and (b) water-in-oil (W/O) (Haji et al., 2022). Targeted delivery of probiotics in the gastrointestinal tract of fish (He, Yang, et al., 2023).	High production complexity and cost (Tan & McClements, 2021). In O/W and W/O, probiotics in direct contact with the water phase, which can increase their chances of inactivation (Gao et al., 2022).
Nanoparticles	These nanoparticles can shield probiotics and improve their stability while also enabling controlled release during the fermentation process (Senthil Kumar & Sheik Mohideen, 2024).	Small size and high surface area-to-volume ratio may lead to potential toxicity (Dube, 2024)
Fluidized Bed Drying	Drying wet particulate and granular materials involves using a fluidized bed dryer, where the probiotic cell suspension is combined with a vibrating bed of absorbers or matrix molecules, facilitating capsule formation through adherence (Poddar et al., 2022; Toledo et al., 2010; Wirunpan et al., 2016).	Fluidized-bed drying resulted in significant reductions in cell viability, likely due to the harsh processing conditions (Broeckx et al., 2016).
Electrospinning	A rapid and continuous drying method performed at room temperature, this process utilizes an electric field to generate a charged jet of a polymer solution. The jet elongates towards a grounded collector under the influence of electrostatic forces, allowing the solvent to evaporate (Hirsch et al., 2021).	Achieving a uniform encapsulation of probiotics remains a challenge, as the process may result in varied fiber structures (Feng et al., 2023).
Coacervation	The colloidal system separates into two distinct liquid phases, with coacervates primarily consisting of the concentrated components and the equilibrium solution phases. (Eghbal & Choudhary, 2018)	Colloidal particles within complex coacervates are unstable, leading to decomposition during storage (Nezamdoost-Sani et al., 2024)
Spray Chilling (Cooling or Congealing)	Creates capsules ranging from 20–200 µm in size, similar to spray-drying, but utilizes cold air for atomization and particle solidification instead of hot air (Koh et al., 2022).	Lower encapsulation capacity and the potential for probiotics to protrude from the beads during storage (Koh et al., 2022)
Fluid Bed Coating	The feed liquid is atomized into a fine spray within a bed of fluidized particles, allowing for efficient encapsulation and drying. This process ensures uniform particle coating and is widely used for encapsulating sensitive materials (Vivek et al., 2023).	High costs and the direct exposure of particles to elevated temperatures, which may lead to particle degradation and potential agglomeration (Agriopoulou et al., 2023).

the health and productivity of aquatic species. Acute stress responses can be beneficial, but chronic stress has detrimental effects, including immune suppression and reduced growth (Mugwanya et al., 2022). Probiotics have shown potential in mitigating the impacts of chronic stress by improving intestinal health and overall resilience (Martínez Cruz et al., 2012; Rahayu et al., 2024). Sustainable management practices, combined with the targeted use of probiotics, can enhance fish welfare and performance.

Advancements in microbial genomics and metagenomics are paving the way for the development of specialized probiotic strains tailored to specific aquaculture systems. These innovations enable the selection of strains with enhanced functional properties, such as improved nutrient synthesis, pathogen resistance, and adaptability to environmental stressors. Incorporating these advancements into industrial-scale production can further optimize the application of probiotics in aquaculture (Abouelela and Helmy, 2024; Fachri et al., 2024).

Economic challenges: Probiotic production for aquaculture is constrained by high costs related to the precise conditions required during fermentation and stabilization processes. Fermentation media, crucial for achieving optimal microbial growth, represent a significant portion of production expenses (Vázquez et al., 2020; Valle-Vargas et al., 2023). Traditional substrates like MRS broth are highly effective but expensive, creating a demand for alternative media (Galante et al., 2023). Thus, the successful use of cost-effective industrial byproducts, such as whey and corn steep liquor, as viable nitrogen sources has gained interest (Salgado et al., 2009; Valle Vargas et al., 2024). These alternatives reduce production costs significantly without compromising biomass quality.

Advanced fermentation techniques, such as fed-batch, batch, and continuous fermentation systems, are critical for scaling up probiotic production. These methods improve cell density and volumetric productivity, enabling industrial-scale operations (Coghetto et al., 2016). However, technical barriers, including infrastructure costs and process optimization for various probiotic strains, limit their widespread adoption (Fenster et al., 2019). For instance, achieving uniform fermentation conditions for strains with diverse nutritional and environmental requirements remains a significant hurdle (Kumar et al., 2022). Stabilization techniques, such as different microencapsulation methods, are essential to maintain probiotic viability during storage and transportation. However, these methods add substantial costs to the production process. Choosing the cost-effective technique while maintaining probiotic viability is an important factor for the production process. Developing cost-effective alternatives for stabilization while maintaining efficacy is a critical area for future research.

Regulatory challenges: The fragmented global regulatory landscape poses significant challenges to probiotic manufacturers. In the European Union (EU), probiotics are regulated under stringent guidelines by the EFSA, requiring comprehensive evidence of safety and efficacy (von Wright, 2005; Koutsoumanis et al., 2021). These standards necessitate detailed strain-specific studies, including assessments of potential antibiotic resistance transferability, which significantly increase the time and cost of market entry (Zavišić et al., 2023).

In the Asia-Pacific region, regulatory standards are inconsistent. Southeast Asian countries, including Indonesia, Malaysia, the Philippines, Singapore, Thailand, and Vietnam, exhibit diverse regulatory approaches for probiotics in foods and health supplements, with only Indonesia, Malaysia, the Philippines, and Thailand enacting specific regulations that include legal definitions and guidelines. While Malaysia, the Philippines, and Thailand publish approved microorganism lists and permit limited generic function claims (Siong et al., 2021). These inconsistent regulations across the region pose challenges for stakeholders and hinder trade and harmonization efforts. This variability complicates market expansion for probiotic producers.

The selection of probiotics for fish is complex, as there is no universally ideal microorganism due to variations in host responses, environmental factors, and intended use (Wuertz et al., 2021). Regulatory challenges stem from the absence of standardized protocols for probiotic selection, with criteria often varying by country and researcher. The selection of probiotics for fish revolves around four main criteria, as shown in Table 4. While a candidate microorganism may not meet all the requirements, adherence to most is considered optimal and advantageous (Caipang and Lazado, 2015).

The successful application of probiotics in aquaculture depends on overcoming technical

Table 4. Selection of probiotics for fish revolves around four main criteria: safety, technological, functional, and physiological aspects. Adapted from (Caipang and Lazado, 2015).

Category	Criteria
Safety	Non-pathogenic to the host and beneficial organisms in the rearing environment
	Free from toxin production
	Correctly identified
Technological	Can be incorporated in diverse forms
	Maintains genetic stability
	Retains desired traits during production, preparation, and storage
Functional	Produces inhibitory compounds
	Enhances digestive physiology
	Stimulates both local and systemic immunity
	Improves rearing conditions
Physiological	Resistant to harsh gastric conditions
	Capable of adhering to and colonizing host surfaces
	Competes effectively with existing natural microflora or pathogens

challenges related to strain stability, production costs, and downstream processing. Continued research into microbial interactions, nutrient optimization, and environmental management will enhance the sustainability and efficiency of aquaculture systems. Integrating probiotics with innovative technologies, such as precision aquaculture and smart monitoring systems, holds the potential to revolutionize the industry while ensuring environmental and economic sustainability.

Conclusion and future directions: The evolving landscape of probiotics in aquaculture necessitates innovative approaches for optimizing production and application at an industrial scale. One critical future direction is the development of tailored probiotic strains through advanced genetic engineering and functional characterization. Recombinant probiotics designed for specific aquaculture species can offer enhanced disease resistance, improved gut health, and optimized nutrient utilization. Leveraging genomic insights and bioinformatics tools will enable the identification of robust strains with multifaceted functionalities.

Probiotics can be incorporated into biofloc systems, which utilize microbial communities to recycle nutrients and reduce waste. This integration not only improves feed conversion efficiency but also minimizes environmental impact, aligning with the growing demand for eco-friendly aquaculture solutions. Additionally, exploring the role of probiotics in mitigating the effects of climate change, such as water quality degradation and temperature fluctuations, can enhance the resilience of aquaculture systems. Advancements in fermentation technologies are paramount. Scaling up probiotic production will require efficient bioprocess optimization, including the use of cost-effective substrates, high-density fermentation systems, and advanced downstream processing techniques. Moreover, encapsulation technologies should be further developed to improve the stability and delivery of probiotics in aquatic environments. Establishing regulatory frameworks is another crucial direction for the future. Probiotic safety and efficacy must be carefully assessed, with clear guidelines to ensure their safe use in aquaculture. A unified regulatory approach across regions can facilitate trade and promote the adoption of high-quality probiotic formulations. By addressing challenges in strain development, scaling industrial production, and establishing regulatory standards, probiotics can significantly enhance fish health, growth performance, and environmental sustainability.

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