Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Review
Volume 11, Issue 6, 2021, 14697 - 14708
https://doi.org/10.33263/BRIAC116.1469714708

Probiotics in Brackish Water Fish Farming: A Special Focus on Encapsulated Probiotics

Ihsana Banu Ishthiaq [1], Jahangir Ahmed [1], Karthikeyan Ramalingam [1,*]

[1] School of Life Sciences, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India
* Correspondence: karthikeyan.sls@crescent.education;

Scopus Author ID 43961552400
Received: 25.01.2021; Revised: 7.03.2021; Accepted: 12.03.2021; Published: 31.03.2021

Abstract: In GMOs' age, pesticides, contaminants, and other anthropogenic practices move towards an environmentally sustainable solution. Aquaculture is one of the fastest-growing food sources connected with several challenges. Aquaculture industries are required to produce food with a better state of health and environmentally friendly. The encapsulation of probiotics in the aquatic feed addresses various problems as many probiotic organisms are shown to strengthen immunity, improve growth, survival, and reproduction of many fish species, including brackish water fish. By including certain probiotics as a food additive, the well-being of aquatic organisms can be enhanced. This review summarizes probiotics' inclusion in an encapsulated form in aquatic feed formulation for enhancing brackish water fish culture.

Keywords: probiotics encapsulation; brackish aquaculture; growth immunity; reproduction.

1. Introduction

The concern to reduce the use of chemicals and transition to environmentally sound practices has increased in recent years. Among the food-producing industries, aquaculture has cropped up as an essential food source for animal protein. Aquaculture is encouraged as it has lower greenhouse gas emissions than any other type of farming; it reduces overfishing and non-selective fishing, preventing under a population of many aquatic species [1-2]. The ultimate goal of the aquaculture industry is to increase productivity sustainably.

Brackish aquaculture is encountering several challenges, such as disease control, health management, survival, and reproduction which are being resolved by applying antibiotics, vaccines, chemicals, medicines, and other supplements. The use of these improvement strategies for better production has caused accumulation, biomagnification, and also the development of antibiotic-resistant pathogens. The use of antibiotics is not preferred as it devastates the gut microbiota flora along with the pathogenic bacteria, suppresses the immunity, and has a detrimental effect on the ecosystem and food safety concerns [3-4]. Thus, increasing need can be satisfied only by practicing brackish aquaculture sustainably with environmentally friendly approaches [5-6]. Moreover, young fish do not have a strong immune system; thus, they do not respond well to vaccines [7-8]. Hence, the alternative option for vaccines is probiotics, and it is live microorganisms that are largely beneficial to the host [9]. The advent of probiotics was an environmentally friendly replacement for antibiotics, chemicals, and pesticides. Probiotic feed improves the aquatic 'animals' gut microbiota and enhances survival, digestion, growth, immunity, resistance to disease, and reproduction when administered appropriately. Fish larvae and fingerlings have an immature digestive system and are poor in digestive capacity. The addition of probiotics to the fingerlings and fish larvae in their feed allows them to overcome this issue.

It has been evidenced that probiotics in the aquatic feed formulation have improved the quality of brackish aquaculture. The delivery mode can be direct addition in the culturing water, as pellets, encapsulating, via live feed by bio-encapsulation and injection [1, 10-11]. Although, encapsulate feed is considered more efficient, it ensures delivery in preferred quantity, protecting the probiotics from the harsh gastric environment or deterioration by water, temperature, and effective carriage into the intestine [12].

The objective of this review is to cover the modes of actions of probiotics in cultivable brackish water fish, including fresh water and marine fish that can also be farmed in brackish water due to their strong ability to withstand variation in salinity [13-14]. The survival, growth, immunity, and reproduction enhancement after introducing probiotics in the feed and preparation of effective probiotics encapsulated feed for the same will be reviewed.

1.1. Mode of action of probiotics.

Probiotics have an overall effect on fish life cycle, as shown in Figures 1 and 3. The digestive system is undeveloped and immature in the larvae and fingerlings of fish. They will not be able to efficiently digest food and consume nutrients, slowing down their growth process. Probiotics release exogenous digestive enzymes and enable the gut zymogens that improve digestion, increases protein availability by proteolysis, release amino acids, vitamins [2]. Probiotics have also been studied to increase the height of villi in the intestine. An increase in villi height successively increases the intestine's surface area in *Oreochromis niloticus* (Nile tilapia) was observed when administered with alginate encapsulated *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* JCM 7255 [15-16]. Effective absorption of micronutrients, survival, and growth was also observed in *Sparus aurata* (Gilthead bream) larvae and fingerlings when bio encapsulated *Bacillus sp.* R2 *and Planococcus sp.* R11 was supplemented [17-18]. Probiotics have been applied successfully to increase the survival, productivity, and decreased mortality of several fish and shrimp species. The immune system is vulnerable in the early life stages of fish and is more susceptible to attack by various pathogens. Probiotics have been widely researched as antimicrobial and can thus be substituted for antibiotics. They prevent pathogenic bacteria's invasion by vying for adhesion sites in the epithelial mucosa of the intestine, nutrition, and the synthesis of antimicrobials. Besides adhesion sites, nutrition probiotics also compete for iron salts called siderophores. Probiotics that dominantly utilize siderophores effectively inhibit the survival of pathogens [19-20]. The 'host's immune system is activated and remains active, which reduced the immune response time; it also acts as an immune modulator and immunostimulant [1]. *Oreochromis niloticus*, *Epinephelus coioides larvae*, *Poecilia latipinna*, *Sparus aurata L.*, and other fishes administered with encapsulated probiotics have expressed enhanced immune system in means of both specific, non-specific immunity and cellular and humoral immunity [21]. The reproduction process was also reported to have increased in fish species after the administration of probiotics. As food absorption and nutrition are vital to any process, so it is for gametogenesis in reproductive. Reproductive health is vital in order to get a good yield. In fish, not all the gamates they produce are fertile due to improper nutrition, maturation, and other factors. Probiotics have ensured the stimulation of genes responsible for reproductive maturity and reproduction [22-23]. The body fat index plays an essential role in the sexual behavior in most species; thus, it is important to balance the dietary unsaturated fatty acids[24-26]. Highly unsaturated fatty acids and group B vitamins were observed to support egg development, provide high rates of energy for vitellogenesis and swamping. It is closely associated with gonadal maturation, which all ines with the onset of puberty. Probiotics have been researched to produce group B vitamins and increase the body fat index. The probiotic *Lactobacillus rhamnosus* has been reported to have a progressive outcome in the spermatogenesis in *Anguilla anguilla* (european eel), vitellogenesis, GSI (Gonadosomatic Index), oocyte maturation, egg quality, fecundity, fertilation rate, hatching rate, sex ratio, embryo survival and over reproductive performance in *Danio rerio* (zebrafish) [27-28]. *Poecilia latipinna* supplemented with bio encapsulated *Saccharomyces cervisiae* was evidenced to increase its average fecundity [27]. *Schilbe mystus* (butter catfish) fed with probiotics were witnessed to have significantly improved quality of gametes, fertilization rate, and hatching rate [29-30].

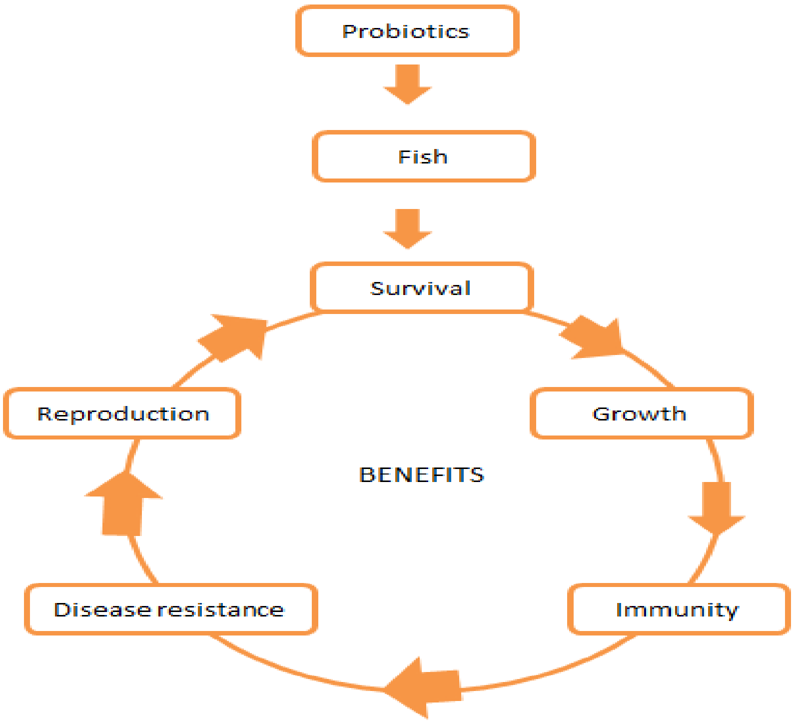

Figure 1. Fish administered with probiotics have been shown improvement in survival, growth, immunity, disease resistance, and reproduction.

1.2. Gut of the fish.

The anatomy of the fish gut is not standard. Some fish possess a pyloric area, gizzard, gall bladder, which may not be present in other fish species. There are also fish species that do not have a stomach. For example, mullets and sea bass have a pyloric area, blenny and goby have a gall bladder, lack stomach. The fish gut plays a predominant role in its immunity [31-32]. Probiotics are associated with fish from their eggs stage. Bacteria are normally found in fish from their early stages. When the fish hatch, they tend to drink water to regulate the osmotic balance. In this process, the bacteria present in the water environment enter their gut and start to colonize [33]. Both probiotics and pathogenic bacteria are among these bacteria. In wild and farmed fish, the colonies of bacteria present differ variously. More aerobic bacterial species and very few or no anaerobic bacterial species can be seen as farmed fish are supplied with sufficient oxygen. Wild fish, on the other hand, have more anaerobic species of bacteria. From their water environment and food, fish derive the bacteria in their gut. The bacterial colonies are also complex since the diets of wild and farmed fish differ. The gut bacteria vary in inter-species also. Moreover, the bacterial species may be present in the stomach or midgut, or even hindgut. Similarly, the bacterial colony density also changes with the portion of the gut where they thrive. The bacterial may also live in a symbiotic relationship in the gut. The gut microbiota can be influenced efficiently to improve fish well-being [31].

1.3. Selection of the probiotic strain.

The selection of probiotics is a critical task when it comes to application. The probiotic strain playing a positive role in one host may not have any effect or can even have a negative impact on another host. Probiotics can be isolated from various areas such as fish gut, terrestrial hosts, water, soil, fermented food, etc. (figure 3). [33]. Bacteria isolated from the healthy gut of poultry chicken have been evidenced as effective probiotics in some fish varieties [34]. The probiotic should be well characterized and identified. The choice of probiotics for fish administration will be most preferred from the fish’s aquatic environment. This is because the probiotics isolated from the native environment appear to have stronger promising characteristics than those extracted from any other environment. Also, the strains purposely introduced have been witnessed to vanish once the administration is withdrawn [35]. Even if the probiotic is isolated from another host, it should adapt and positively affect the new environment [36].

Resistance to the harsh pH and bile juice, the potential to bind to the mucosal surface of the intestine, a beneficial character to the host such as hindering the pathogenicity by competing with the pathogen or by synthesizing inhibitory compound, aiding digestion, functioning as an immunomodulant, etc., are the key characteristics expected in selecting a probiotic. The probiotics should also be tested, plated, or cultured *in vitro* conditions for commercial purposes, able to withstand preservation [6, 37]. The characterization should be done by sequencing the 16sRNA. The antibacterial activity should be tested with various pathogens with agar well, and the spot lawn technique [28], viability, and other properties change after storage encapsulation should be checked in vitro. More importantly, the probiotic used should not be pathogenic to the host [38].

2. Encapsulation

Encapsulation is the idea of immobilizing or enclosing probiotics in a capsule which may be a live prey or a polymer (Figure 2) for effective, confirmed delivery and controlled release [39-41]. When live prey is used, mainly rotifers, Artemia, and copepods, it serves a dual purpose [42]. The probiotics increase the prey’s nutritive value [43-44] and enhance the fish's living (Table 1). The bio-encapsulated feed cannot be stored but has to be prepared instantly. Bio-polymer encapsulation involves the microencapsulation of probiotics into small oral beads made up of alginate, chitosan, agar, etc. [45]. These ensure the storage and viability of the probiotics for quite a long term [46]. The bacterial density for encapsulation was maintained between 10^6 to 10^{10} CFU/ml [47] when the bacterial load was increased in tor, but larvae exhibited a lower feeding rate [48].

2.1. Bio-encapsulation.

Bio-encapsulation involves the incorporation of products into live prey, enriching them with products in order to deliver them to the host. Due to their small size, optimal nutrition, and ease in digestion, rotifers and Artemia are the most common life feed for fish larvae. These organisms can also be used to deliver probiotics to the fish in bio-encapsulated form. These organisms take up the bacteria by absorption or filtration of particles into their digestive tracks and exoskeletons in 48 to 10 hours. Encapsulating *Bifidobacterium animalic* and *Lactobacillus johnsonni* in arteria showed an increase in growth in *Chirostoma jordani* larvae (charal fish) in the early 30 days. However, a significant difference was noticed only after 60 and 90 days of feeding. The group of fish fed with *Lactobacillus johnsonni* presented slightly higher growth than the other [49]. Administration of sea bream larvae with bio encapsulated Planococcus to rotifers and Artemia significantly improved larval survival, length, weight, specific growth, protein, and lipid nutritional value. The protease activity, the number and length of the villi, and the number of goblet cells were significantly increased, suggesting an improvement in digestion and absorption. As the mode of distribution shifted, the range of growth varied significantly. Larvae fed with only live bio encapsulated probiotics had the greatest improvement compared to rearing and bio encapsulated plus rearing [17]. Molly fish (*Poecilia latipinna*) fed with Artemia enriched with *Saccharomyces cervisiae* reported enhancement in growth, survival, reproduction in terms of average fecundity, lysozyme activity, and immunity [27]. Although the impact of encapsulated probiotics is not yet advanced in the reproduction of brackish water fish. Rotifers treated with lactic acid bacteria were demonstrated to improve turbot larvae's growth and immunity [50].

2.2. Microencapsulation.

Microencapsulation involves the encapsulation of probiotics in bio-polymer matrices that are derived from sea algae, plants, or animals. Probiotics encapsulated using this technique are immobilized and targets the delivery of life probiotics [10]. This bio-polymer matrice is mostly non-nutritive and may act as an immunostimulant [15, 27,].

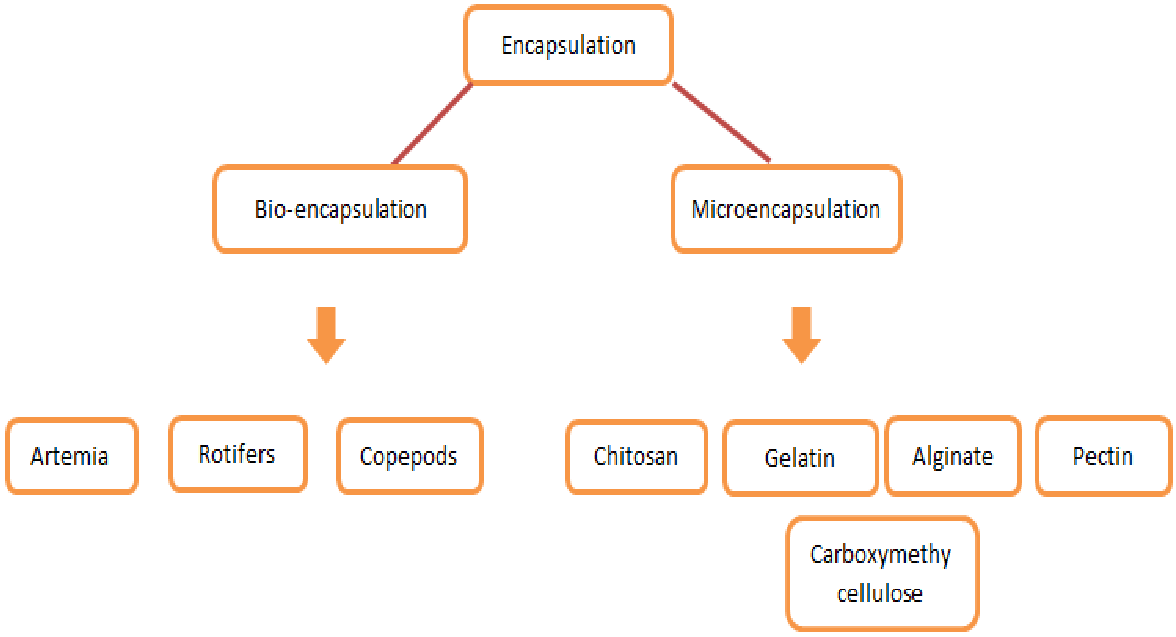

Figure 2. Various forms of encapsulations of probiotics as fish feed additives.

A study reported that the alginate of the microencapsulated beads had an immunostimulatory effect and served as a prebiotic in fish [51-52]. Unlike freshwater fish, a microencapsulated feed can be efficiently digested by marine fish or brackish water fish [50].

These capsules release the probiotics gradually into the gut [53]. Microencapsulation provides the advantage of encapsulating other feed and probiotics such as plant extracts, lipids, proteins, etc. in appropriate compositions as a source of prebiotics [54-55]. Prebiotics are compounds that benefit the probiotics and also the host acting as immunostimulants [56]. In calcium alginate encapsulation, a higher concentration of calcium ion has negatively affected bacterial viability. Hence, a calcium chloride concentration of less than 3% was preferred. Microencapsulation also has the flexibility to produce capsules of desired and determined size, shape, thickness, and bacterial density. These capsules are resistant to the stomach's pH, withstand temperature, and appropriate for storage and transport [7, 46].

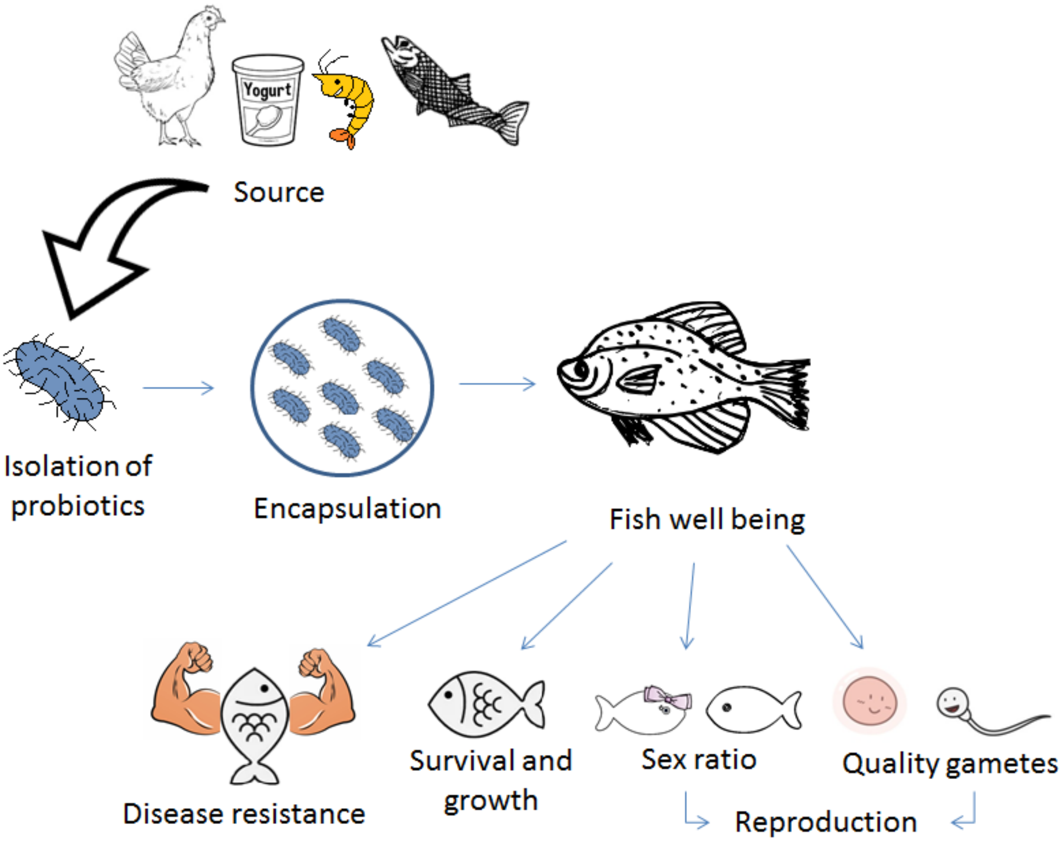

Figure 3. Well being of the fish in terms of reproduction, survival, and growth, disease resistance after applying encapsulated probiotics isolated from various sources.

Table 1. Benefits of various encapsulated probiotics on brackish water farmed fishes.

SI. No.	Probiotic strain	Host	Encapsulated form	Benefit	Reference
1.	*Lactobacillus rhamnosus*	*Oreochromis niloticus*	Alginate encapsulation	Increased growth, higher villi height, acidophilic granulocytes, intraepithelial lymphocytes and mucous cells, Resistance against *S. agalactiae*	[16]
2.	*Lactobacillus rhamnosus*	*Oreochromis niloticus Juveniles*	encapsulated with fat vegetable matrices	Increase in body weight and length, reduced potential pathogens, up-regulation of npy, agrp, and ghrelin and the decrease of leptin increasing the feeding rate	[57]
3.	*Saccharomyces cerevisiae*	*Oreochromis niloticus*	Alginate encapsulation	Increased weight, specific growth rate, Resistence against *Streptococcus agalactiae*, increased villi height, acidophilic granulocytes, intraepithelial lymphocytes and mucous cells	[15]
4.	*Bacillus sp.* NP5	*Oreochromis niloticus*	Maltodextrin encapsulation	Resistance against *Streptococcus agalactiae*, increased Hb, Ht, and EC	[47]
5.	*Saccharomyces cerevisiae* JCM 7255	*Oreochromis niloticus*	Alginate encapsulation	Resistance against *Streptococcus agalactiae*, increased villi height, acidophilic granulocytes, interaepithelia lymphocytes and mucous cells	[58]

SI. No.	Probiotic strain	Host	Encapsulated form	Benefit	Reference
6.	*Bacillus clausii* DE5 *and Bacillus pumilus* SE5	*Epinephelus coioides larvae*	Bio-encapsulated in copepods(*Pseudodiaptomus annandalei)*	Increase in body weight, survival, increased AKP activity, Lysozyme activity	[59-60]
7.	*Bacillus subtilis, Bacillus licheniformis and Bacillus pumilus*	*Sparus aurata larvae*	Bio-encapsulated in Artemia and rotifer	Increase in body weight and standard length, lower HSP70 gene expression levels reduced GR levels	[61]
8.	EcoPro probiotic	*Rachycentron canadum larvae and fingerlings*	Bio-encapsulation in Artemia and rotifer	Increased survival and production	[62]
9.	*L. delbrueckii delbrueckii (AS13B)*	*Dicentrarchus labrax*	Bioencapsulation in rotifer and Artemia	Increased standard length, body weight, IGF1 mRNA, decreased MSTN mRNA	[63]
10.	*Shewanella putrefaciens Pdp11*	*Sparus aurata L.*	Alginate encapsulation	Increased serum peroxidase activity, upregulation of mhcIIa and tcrb, increased serum IgM leve, enhanced innate immune	[45]
11.	*Bacillus sp.* R2 *and Planococcus sp.* R11	*Sparus aurata*	Bio-encapsulation in Artemia and rotifer	Improved larval survival, length, weight, specific growth, protein and lipid nutritional value	[17]
12.	*Pediococcus acidilactici and Saccharomyces cerevisiae*	*Pollachius pollachius larvae*	Bio-encapsulation in Artemia	Promoted growth	[64-65]
13.	*B. clausii* DE5 *and B. pumilus* SE5	*Epinephelus coioides larvae*	Bio-encapsulation in copepod	Increased body length and weight, resisted pathogenic Vibrio sp. growth	[59]
14.	*Saccharomyces cervisiae*	*Poecilia latipinna*	Bio-encapsulation in Artemia	Enhanced growth, survival, reproduction in terms of average fecundity, lysozyme activity, and immunity	[27]
15.	Bacterial strain 4:44 and PB52	*Scophthalmus maximus L.*	Bio-encapsulated in rotifers	Higher resistance to stress or infection by pathogens	[48]
16.	*Vibrio* strains, PB 1-11 and PB 6-1	*Hippoglossus hippoglossus*	Bio-encapsulated in Artemia	Inhibition of a pathogenic Vibrio	[66]
17.	*Lactobacillus* spp.	*Sparus aurata, L.*	Bio-encapsulation Rotifera and Artemia	Increase in specific growth rate (SGR) and survival, enhanced digestive enzyme activities	[67]
18.	EC5	*Scophthalmus maximus*	Bio-encapsulated in Artemia and rotifers	Better survival and mortality rate against AHL molecules	[68]
19.	*Lactococcus lactis subsp. Lactis*	*Dicentrarchus labrax larvae*	Bio-encapsulated in Artemia	Resistance against Vibrio anguillarum, better survival and mortality rate	[69]
20.	*Pediococcus acidilactici*	*Psetta maxima*	Bio-encapsulated in rotifer	Positive prevention in colonization	[70]
21.	Probiotic Alken Clear-Flo® 1006 (ACF-1006)	*Rachycentron canadum*	Bio-encapsulated in rotifer	Elimination of undesired bacteria, enhancement in health	[71]

3. Encapsulated and Non-Encapsulated Evaluation

Encapsulated probiotics have been tested to be more effective than administration via non-encapsulation. Sea bream larvae group administered with encapsulated probiotics (*Bacillus subtilis, Bacillus licheniformis,* and *Bacillus pumilus*) form alone have shown higher body weight, total length, and IGFI gene expression than group administered with encapsulated probiotics and added to rearing water [61]. Another experiment with Seabream larvae also demonstrated the effectiveness of encapsulated probiotics in which the control group and probiotics added to the rearing tank alone had no significant difference. The encapsulated probiotics (*Lactobacillus* spp.) showed notably better results in terms of SGR and survival, enhanced digestive enzyme activities [67]. This outcome of encapsulated probiotics may be due to the probiotics' escaping from the gastric tract's severe pH, assured delivery with an optimal concentration of the probiotics via encapsulation. Furthermore, encapsulated beads expressed thermal resistance than free probiotic (L. acidophilus) cells where encapsulated cells reduced by 1.99 log cycles and free celled reduced by 5 log cycles when open to 60 ^{o}C for 30 minutes [72-73]. Encapsulated chitosan double-coated bead expressed relatively higher heat tolerance (3 to 4 log CFU/ml viability) even at 90 ^{o}C for 5 minutes than alginate encapsulated and non-encapsulated probiotics (no viability) [12].

4. Conclusion

Application of probiotics in brackish fish culture ambitions to promote food source with nutrition and safety. Probiotics are present in the gut of aquatic and terrestrial animals. Since ancient times, probiotics have been studied about their benefits, mode of action, and their supplementing them as a food additive. The introduction of probiotics is an environmentally safe and sustainable method. Encapsulated probiotics offer better results than non-encapsulated forms of application. When administered in encapsulated form, probiotics assure the delivery in the desired concentration by protecting the probiotics from devastating environments. Moreover, microencapsulation is benefits storage for commercial purposes. In brackish aquaculture, the probiotic dosage used may vary between 10^6 to 10^{10} CFU/ml depending on the expected result and fish species. Probiotics have enormously positive effects on cultivated fish's well-being, such as digestion and absorption, survival and growth, immunity and disease resistance, reproduction. Thus remarkably improves the key goals- production and nutritive value in aquaculture sustainably.

Funding

This research was supported by the DST Science and Engineering Research Board (SERB), and the Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) India.

Acknowledgments

The authors declare no acknowledgments.

Conflicts of Interest

The authors declare no conflict of interest.

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