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REVIEWS OPEN ACCESS

The Application of Prebiotics, Probiotics, Synbiotics, Postbiotics and Parabiotics in Aquaculture

Severus R. Wisastra[1†], Dyah Wulandari[1,4†*], R. Haryo B. Setiarto[2,3], Anto Budiharjo[4,5], Chumporn Soowannayan[6], M. N. Lani[7], A. H. Condro Haditomo[8]

[1] Department of Food Technology, Faculty of Agricultural Technology, Soegijapranata Catholic University, Semarang, Central Java, Indonesia
[2] Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia
[3] Research Collaboration Center for Traditional Fermentation, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia
[4] Molecular and Applied Microbiology Laboratory, Central Laboratory of Research and Service, Diponegoro University, Semarang, Central Java 50275, Indonesia
[5] Biotechnology Study Program, Faculty of Science and Mathematics, Diponegoro University, Semarang, Centrak Java 50275, Indonesia
[6] Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, 10400, Thailand
[7] Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[8] Aquaculture Department, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java 50275, Indonesia
*Corresponding author: dyahwulandari@unika.ac.id † : These authors contributed equally to this work.

ABSTRACT

The aquaculture industry has been growing rapidly in the last decades and has become one of the main producers of aquatic organisms. There has been increasing interest in using biotic supplements, prebiotics, probiotics, synbiotics, postbiotics, and parabiotic as alternatives to antibiotics and chemical treatments in aquaculture. These biotic agents hold promises for improving the gut health, growth performance, reproductive system, immune response, and disease resistance of aquatic organisms. This review delves into the mechanisms, benefits, and applications of biotic supplements in aquaculture systems. It highlights the growing interest in postbiotics and parabiotics, which, unlike probiotics, do not require live bacteria to deliver health benefits. However, despite their potential, postbiotics and parabiotics are still rarely used in aquaculture. Growth enhancement is often linked to an increase in digestive enzyme activity or the stimulation of host appetite-regulating hormones. Additionally, biotic supplementation can also act as ammonia-oxidizing and nitrite-oxidizing agents to maintain appropriate water quality. They also improve reproductive performance in aquatic species, aiding in broodstock maintenance and enhancement. Moreover, biotic supplements boost innate and adaptive immune responses, helping to combat bacterial diseases in aquatic environment. Furthermore, the review addresses the safety and challenges of integrating.

Keywords

aquaculture, biotic supplements, sustainable

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1. Introduction

Indonesia's marine and fisheries industry plays an important role in national food production and economic development [1, 2], often referred to as the Blue Economic Sector. This sector is expected to be the driving force for national food security and development. Nevertheless, exponential population growth contributed to increased food needs. Additionally, wild capture fisheries sectors are facing overfishing and are nearing their capacity limit, resulting in declining production [3]. New techniques for farming aquatic organisms are being introduced to address this issue. Aquaculture is a farming technique that grows aquatic organisms under controlled conditions. This farming technique also allows a selective increase of specific species.

According to data by Food and Agriculture Organization (FAO) [4], Indonesian aquaculture has been experiencing annual growth of up to 13.65% from 2000 to 2021 and ranked first in Southeast Asia. FAO report in 2024 [4] shows Nile tilapia, catfish, milkfish, carp, and white-leg shrimp dominate the aquaculture industry in Indonesia. In response to growing demand, aquaculture has adopted intensive practices, which involve maintaining a high density of organisms. However, this approach can lead to increased stress and degraded water quality, compromising animal welfare and increasing their susceptibility to diseases and infections, ultimately resulting in elevated mortality rates [5] Moreover, the large quantities of organic waste produced can cause considerable environmental harm [5, 6, 7].

The fast growth of aquaculture is humbled by the inevitable outbreak of bacterial, viral, fungal, and parasitic diseases that resulted in economic losses. In Indonesia, it is reported that a total of around $3.3 million was lost due to fish diseases [8]. To prevent the occurrence of disease outbreaks, prevention and control of aquatic organisms have focused on the use of chemicals and antibiotics, which exert a negative impact on public health through the generation of residual waste and antibiotic-resistant strains [9, 10, 11]. Exploring alternative approaches to reduce infection occurrence in aquaculture without antibiotic properties while also maintaining a high output has become a promising and effective solution.

The probiotic term has been widely used to describe live bacteria that have a beneficial effect with appropriate amounts and remain alive when reaching the gastrointestinal tract [5, 12, 13]. Probiotics play an important role in maintaining microbial balance within the host, improve the beneficial intestinal microbial populations, and increase digestive enzyme activities, which help improve nutrient absorption while protecting the host from pathogenic bacteria and modulating the immune system [14]. Additionally, these approaches are less hazardous compared to conventional chemical treatment [15]. Various probiotic strains, including *Lactobacillus*, *Bacillus*, *Pseudomonas*, and *Pediococcus*, are commonly used in aquaculture [16, 17]. To further support probiotic survivability and activity, prebiotics, a non-digestible food ingredients that stimulate the growth of beneficial microbes are often included, leading to the development of synbiotics [18]. Surprisingly, there is a report that stated that microbial viability is not necessary to produce benefits in the host [19]. This information has directed attention towards, postbiotics and parabiotics, which refers to metabolic products and inactivated microbial cells, respectively. Moreover, these biotic components can reduce the possibility for probiotic to become a reservoir for resistance gene [20]. Although probiotics have been extensively explored, the roles and comparative advantages of other biotics are gaining increasing attention in aquaculture. Therefore, this review aims to examine the use of these five biotic supplements in aquaculture, the actual concept of use, production, and further research prospects. Ultimately, this review seeks to provide insights into how these biotics can be strategically implemented to improve aquatic animal health, enhance sustainability, and guide future research directions in aquaculture.

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2. History and definition of prebiotics, probiotics, synbiotics, postbiotics and parabiotics

The International Scientific Association for Probiotic and Prebiotics (ISAPP) defines the term probiotic as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [21]. The main challenge when administering probiotics to the host is maintaining their viability. Moreover, probiotics are sensitive to heat and acid. To address this challenge, researchers try to incorporate prebiotics together, which develop the concept of synbiotics. Glenn Gibson and Marcel Roberfroid in 1995 proposed that prebiotics were non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of certain beneficial bacteria in the colon [22], before being updated to current definition (Table 1). This early research laid the groundwork for exploring how dietary components could directly influence the composition and function of the gut microbiome. Nowadays, prebiotics have expanded to not only plant-derived oligosaccharides but also several polysaccharides, like resistant starch and pectin [23, 24]. The primary function of prebiotics is to act as a food source for the beneficial bacteria in the gastrointestinal tract, promoting their growth and activity.

Not until recently has it been found that the viability of the microorganism is not necessary to give the beneficial effect [19, 25]. As such, metabolites derived from probiotics, or their non-viable cells become a favorable and promising alternative to give beneficial effects known as postbiotics or parabiotics. Currently, the term postbiotics is defined by ISAPP as the “preparation of inanimate microorganisms and their components that confers a health benefit to the hosts”. However, others also try to define postbiotics in different terms which only highlight probiotics’ byproducts and metabolites without the inanimate microogranism [26, 27, 28]. Table 1 summarizes the definition for biotic supplements.

Table 1: Definition of prebiotics, probiotics, synbiotics, postbiotics and parabiotics

Term	Definition	Examples	Ref
Probiotics	Live microorganisms that confer health benefits when consumed in adequate amounts.	*Lactobacillus acidophilus*, *Bifidobacterium bifidum*	[21]
Prebiotics	Selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.	Inulin, Fructooligosachharide (FOS), Galatooligosachharide (GOS)	[29]
Synbiotics	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host.	*Lactobacillus* + inulin	[18]
Postbiotics	Non-viable bacterial products or metabolic products from microorganisms that have biological activity in the host.	Short-chain fatty acids (SCFAs), bacteriocins	[28]
Parabiotics	Non-viable microbial cells (either intact or broken) or crude cell extracts which when administered in adequate amounts, confer a benefit on the host.	Heat-killed *Lactobacillus*	[28]

The use of biotic supplements in animals, particularly as microbial feeds or additives for aquatic species, dates back to 1986, when Kozasa first introduced the concept using the spore of *Bacillus toyoi* to increase the growth of yellowtail fish (*Seriola quinqueradiata*) and reduce the mortality of Japanese eel infected with *Edwardsiella* sp. [30]. Compared between terrestrial and aquatic animals, the presence of probiotics and other microorganisms in aquatic

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animals is transient due to the constant flow of water. This poses a positive and negative impact; as the water flows, both probiotic and pathogen bacteria can transmit or adhere to other organisms. Additionally, water is an ideal environment for pathogen bacteria growth, thus making the use of probiotics more complex and requiring constant use since the environment suffers periodic changes, from salinity, temperature, and dissolved oxygen (DO) that can alter the dominant microorganism species present [31].

3. Production of probiotics, synbiotics, postbiotics and parabiotics

The production of commercial probiotics (Figure 1) aims to achieve the highest possible yield, stability, and consistent performance. The process begins with a pure, contaminant-free stock of probiotics, followed by a fermentation process using suitable media. The probiotic cells are then concentrated through centrifugation and may undergo freezing steps to preserve their viability and extend shelf life [32]. In the production of synbiotics, oligosaccharides or other prebiotics are added alongside the probiotic cells [33]. The production of postbiotics is similar to the preparation of probiotics, as both involve a metabolic process by bacteria that results in the production of various metabolites. However, in postbiotics preparation, it is essential to eliminate the remaining bacteria and retain the metabolites only. Microbial cells can undergo several processes to disrupt the structure of cell membrane to facilitates the release of intracellular metabolites into the extracellular medium [34]. Common methods used include sonication, heat treatment, pulsed-electric field, and high pressure [34]. The choice of method depends on the specific bacterial strain and the sensitivity and stability of the metabolites under different conditions. Additionally, centrifugation and microfiltration are often done to ensure complete removal of living cells. However, for postbiotics that are secreted by live cells and already present in the extracellular medium, centrifugation and microfiltration steps are typically sufficient [35, 36, 20]. Parabiotics, on the other hand, are probiotic microorganisms that have undergone an inactivation process. Various methods can be used for inactivation, including heat treatment, chemical exposure, and irradiation, with heat treatment being the most commonly applied approach [28]. As a result of this process, inactivated probiotics can yield two distinct types of biotic products: postbiotics, which utilize only microbial metabolites and parabiotics, which make use of the non-viable microbial cells themselves to deliver health benefits.

In a recent study, it is popular to utilize agro-industrial effluents as media for the growth of probiotic bacteria since they are cheaper and more abundant compared to synthetic media. Using cheese whey effluents able to produce *Bifidobacterium lactis* BB12A postbiotics, including exopolysaccharides and conjugated linolenic acid bacteriocins up to 160 µg/l, 105 µg/ml, respectively, it also shows antimicrobial properties [37]. The use of agro-effluent activity as a medium is also similar to that of synthetic media. A study by Dominguez [38] found that using soybean protein (20 g/L) resulted in the production of cerein 8A (*Bacillus cereus* bacteriocin) of 1600 AU/ml, which is similar to brain and heart infusion broth (BHI). The need for certain media and nutrients for maximum bacteriocins production depends on the specific type of bacteriocins. Some amino acids can be self-produced by the bacteria, and some require external supplementation [39]. A study by Yi *et al.* [40] found that the addition of external amino acids, glycine and cysteine, stimulated the production of lac-B23 bacteriocin from *Lactobacillus paracasei* J23 by 250% and 325%, respectively. From an economic standpoint, by doing so, the commercialization of bioactive compounds can be profitable for the biotechnology industry [41].

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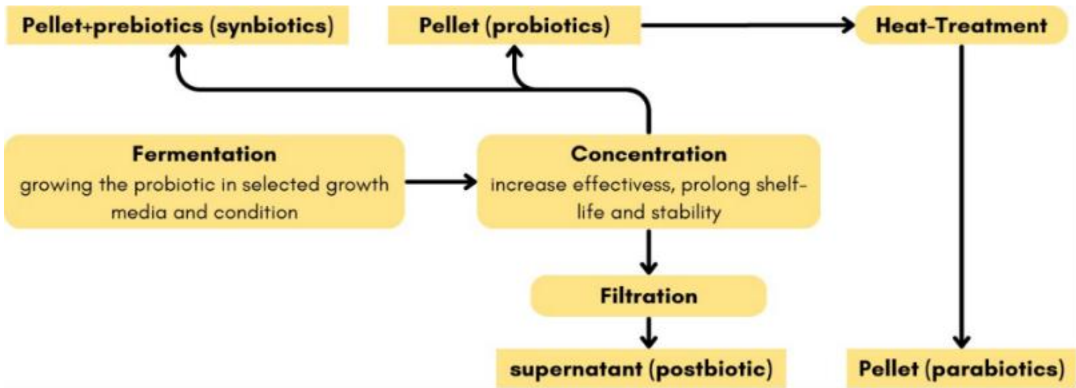

Figure 1: Classification and production of several biotic supplement

4. Prebiotics, probiotics, synbiotics, postbiotics and parabiotics selection criteria

Over the past century, since the discovery of *Lactobacillus bulgaricus* by Stamen Grigorov in 1905 [42], researchers have continuously worked to isolate and identify new probiotics strain. Advances in DNA sequencing helps to extend the probiotic taxa, uncovering strains with novel beneficial properties [43, 44, 45]. However, these novel strains are rarely used compared to traditional probiotics, due to their safety concerns and costly procedures [43, 44, 45]. To be classified as probiotic, a microorganism must undergo rigorous evaluation, including stress tolerance, adhesion ability, anti-pathogenic activity, safety assessment, gemone sequencing, and clinical trials, which can be costly. Only those with strong functional properties and no adverse effects on the host are selected [46]. Prebiotics, on the other hand, must positively impact gut health, are stable under various conditions, and are resistance to digestion in the upper gastrointestinal tract, can be fermented by gastrointestinal microflora. They should recognized as Generally Recognized As Safe (GRAS), with no significant adverse effects [29, 47]. Selecting synbiotics requires careful selection of prebiotic and probiotic ensuring both works complementarily or synergistically. The prebiotic should support the chosen probiotic strains' growth and remain stable during processing and digestion [18, 29]. Ideally, the combination should show superior effectiveness to either component alone.

Postbiotics, which consist of bioactive compounds produced by probiotics should have a well-characterized composition, including beneficial microbial byproducts like short-chain fatty acids (SCFAs) or peptides, and be free from contaminants, with the progenitor microorganisim are already molecularly charaterized. The mechanisms of inactivation must be confirmed, and their safety and health benefits should be assesed and well-researched [48]. Similary, parabiotics, which are inactivated microorganisms that still confer health benefits, must ensure health benefits. The chosen strain should retain its beneficial properties after inactivation (e.g., through heat or pressure). The inactivation method should be standardized to preserve bioactive components, and the parabiotic should interact effectively with the host's cells or microbiota. It also must be safe and stable during storage [49].

5. Application of probiotics, prebiotics, synbiotics, postbiotics and parabiotics in aquaculture

The main usage of probiotics in aquaculture started as a growth promoter. However, new findings about its beneficial effects have been found, including as an immune system and host pathogenic bacteria resistance enhancer

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and reproduction inducer. The application of biotic supplements in aquaculture can be done by several methods, including mixing them directly with their feed or directly add them to the water.

5.1 Promote growth and nutrient digestibility enhancement

Biotic supplements have been used in aquaculture mainly for their properties that enhance aquatic organism growth (Table 1). Researchers show adding biotic supplement in animal feed as an additive can increase animal's final weight and reduce the feed conversion rate (FCR), indicating a better and more economical feed. Although some studies also found no significant effect on the addition of probiotics to the feed [50], this varying result can depend on the concentrations of biotic supplement and animal stage life. For example, a study conducted by Haque [51] on Pangasianodon hypophthalmus found that feeding with probiotics during the larval to fry stage results in a higher growth rate in the fry growth stage compared to fry to fingerlings. The exact mechanisms through which biotic supplements enhance growth remain unclear. One factor is linked to the host's appetite, while the other involves the host's capacity to digest nutrients and produce enzymes.

Vertebrate appetite is controlled by orexigenic (stimulate) and anorexigenic (inhibit) factors within the brain, which receive information from the gastrointestinal tract and peripheral nervous system [52]. Research by Gioracchini et al. [53] found that the mixture of encapsulated freeze-dried *Lactobacillus rhamnosus* IMC 501® in 10^6 CFU/g can induce tilapia (*Oreochromis niloticus*) appetite hormones, neuropeptide Y (NPY), agouti-related protein (AgRP), and ghrelin, while suppressed leptin hormone that gives satiety feeling. The probiotic group was significantly lower in secretion of leptin, while NPY, AgRP and ghrelin was significantly higher from the control at a p-value of 0.0001 after 30 days of feeding. Besides that, the insulin-like growth factor (IGF), IGF-1, IGF-2, and IGF-1 receptor also showed an increase in the probiotic group. Another study also found growth-promoting properties in using Mandarin fish (*Siniperca chuatsi*) as hosts. It was found that the supplementation of *Lactobacillus plantarum*, *L. rhamnosus* or *Clostridium butyricum* at the concentration of 10^8 CFU/ml in feed pellet increased the NPY and AgRP genes and decreased leptin secretion, with no effect on ghrelin was observed [54]. Similarly, a study using prebiotics only, β-glucan, GOS, and mannanoligosaccharides (MOS) shows increased growth in *Channa striata*, however compared to the probiotic group, *Saccharomyces cerevisiae* and *L. acidophilus* the growth enhancement is still lower [55]. Additionally, studies on parabiotics showed results similar to those of probiotics, using heat-killed *Bacillus* sp. SJ-10 with a concentration of 3.34×10^8 CFU/ml in *Paralichthys olivaceus* induced the secretion of growth hormone and IGF-1 expression [56]. Another factor that is believed to be related to growth enhancement is an increase in digestive enzymes, such as protease, amylase, lipase, and cellulose [57]. Probiotic strains can synthesize extracellular enzymes, thus making nutrient absorption more effective. A study on the use of *Lactobacillus pentosus* AS13 as a dietary probiotic for *Litopenaeus vannamei* at the concentration of 10^7 CFU/g found that they can secrete digestive enzymes, such as protease and cellulase and increase their activity [58].

Table 2: The effect of potential biotic supplements on aquatic organism growth performance and growth hormone

Biotic Supplement	Dose	Host	Duration (each day)	Result	Ref
Probiotics					
Bacillus coagulans	106 CFU/g	*Ctenopharyngodon idella*	60 days (2x)	↑FBW, ADG, RGR, DE ↓FCR	[57]
Lactobacillus acidophilus					
Rhodopseudomonas palustris					

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Biotic Supplement	Dose	Host	Duration (each day)	Result	Ref
Bacillus subtillis	4 x 106 CFU/g	*Cyprinus carpio*	180 days	↑FCE, SGR, FI ↓FCR	[59]
Bacillus velezensis and Lactobacillus plantarum N11	107, 108 CFU/g	*Oreochromis niloticus*	30 days (2x)	↑FBW, WG, SGR ↓FCR	[60]
Bacillus licheniformis	105, 107 CFU/g	*Oreochromis mossambicus*	28 days (2x)	↑FBW, SGR ↓FCR	[61]
Clostridium butyricum	1.2 x 106 CFU/g	Hybrid tilapia (*O. niloticus* x *O. aureus*)	56 days (3x)	↑FBW, WG, SGR PR, LR ↓FCR	[62]
B. velezensis AP193	4 x 107 CFU/g	*Ictalurus punctatus*	70 days	↑WG =FCR	[63]
B. subtilis 7k	106, 108, 1010 CFU/g	hybrid Hulong Grouper (*Epinephelus fuscoguttatus* x *E. lanceolatus*)	14 days (2x)	↑FBW, WG, ADG, FE	[64]
Chromobacterium aquaticum	107 CFU/g	*Danio rerio*	8 weeks (2x)	↑GK, HK1, G6Pase, PK-L, GHr, IGF-1	[65]
Bacillus thuringiensis QQ1	109 CFU/g	*Lates calcarifer*	5 weeks (3x)	↑FBW, FTL, RGR, SGR, PER, FER, FI, DE ↓ FCR	[66]
Bacillus cereus QQ2					
B. subtilis (AQUA-GROW®)	0.5, 1, 1.5 g/kg	*L. vannamei*	56 days (3x)	↑FBW, WG, SGR, FI, ADG ↓ FCR	[67]
L. acidophilus powder	0.1 g/kg	*Channa striata*	16 weeks (3x)	↑WG, RG, SGR, FE, PER ↓FCR	[55]
Saccharomyces cerevisiae	10 g/kg				
Prebiotics					
β-glucan	2 g/kg	*C. striata*	16 weeks (3x)	↑WG, RGR, SGR, FE, PER ↓FCR	[55]
GOS	10 g/kg				
MOS	5 g/kg				
Xylooligosaccharide (XOS)	50 g/kg	*O. niloticus*	84 days (2x)	↓FCR =BMI	[68]
GOS					
XOS + GOS	25 g/kg each				
XOS with plant diet	10 g/kg	*Dicentrarchus labrax*	7 weeks (6 days a week)	↑WG, FBW =FE, PER, FI	[69]
MOS	6 g/kg	*Pangasianodon gigas* × *P. hypophthalmus*	10 weeks (2x)	↑FR, PER, DE ↓FCR	[70]

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Biotic Supplement	Dose	Host	Duration (each day)	Result	Ref
Arabinoxylan-oligosaccharide (AXOS) + inulin	40 g/kg each	*L. vannamei*	8 weeks (4x)	↑FBW, ADG, SGR, DE ↓FCR	[71]
GOS	5 g/kg	*Acipenser baerii* × *A. schrenckii*	56 days (2x)	↑FBW, WG, SGR ↓FCR	[72]
Synbiotics					
Corn Cob derived XOS + *L. plantarum* CR1T5	10 g/kg; 108 CFU/g	*O. niloticus*	12 weeks (2x)	↑FBW, WG, SGR ↓FCR	[73]
GOS + Enterococcus faecalis	10 g/kg; 6.9 x 107 CFU/g	*Astacus leptodactylus*	126 days (3x)	↑FBW, SGR , PER, DE ↓FCR	[74]
MOS + E. faecalis					
β-glucan + Pediococcus acidilactici	0.5 g/kg; 5x107 CFU/g	*L. vannamei*	90 days (5x)	↑FBW, WG	[75]
β-glucan + *B. subtilis*				↑=FBW, WG	
Alginate oligosaccharides + *B. licheniformis* WS-2	10g/kg; 109 CFU/g	*A. leptodactylus*	60 days (1x)	↑WG, SGR, DE ↓FCR	[76]
FOS + E. faecium (Biomin IMBO)	0.5, 1, 1.5 g/kg	*Oncorhynchus mykiss*	60 days (3x)	↑FBW, WG, SGR ↓FCR	[77]
Postbiotics					
S. cerevisiae cell wall extract (Megamos®) and freeze-dried L. bulgaricus powder	4 and 2 g/ kg	*Mugil capito*	60 days	↑FBW, WG, SGR, FI, PER, PPV ↓FCR	[78]
L. plantarum cell-free extract	10 ml/ kg	*L. vannamei*	15 days (4x)	↑FBW, WG, SGR, DE ↓FCR	[79]
L. plantarum fermentation supernatant					
B. subtilis LCBS1 cell free extract	8 x 107 CFU/ml	*Lithobates catesbeianus*	8 weeks (2x)	↑FBW, WG, PE ↑=DE	[80]
B. subtilis LCBS1 cell free supernatant				↑=FBW, WG, DE =PE	
Bacillus, Lactobacillus, Saccharomyces cell lysate	20 g/kg	*Macrobrachium nipponense*	8 weeks (2x)	↑FBW, WG, SGR	[81]
Parabiotics					
Heat-killed (HK) *Bacillus sp. SJ-10*	3.34 x 108 CFU/ml	*Paralichthys olivaceus*	8 weeks (2x)	=FBW, WG, SGR, FCR ↑IGF-1 expression	[56]
HK *L. plantarum*					
HK *B. subtilis LCBS1*	8 x 107 CFU/ml	*L. catesbeianus*	8 weeks (2x)	↑FBW, WG, PER, DE	[82]
HK *P. pentosaceus PP4012*	106 CFU/ g	*L. vannamei*	12 weeks (2x)	↑FBW,SGR, FE	[83]

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Biotic Supplement	Dose	Host	Duration (each day)	Result	Ref
HK *B. cereus BR2*	1010 CFU/ml	*Clarias sp.*	30 days (3x)	↑FBW,SGR ↓FCR	[84]
HK *B. pumilus SE5*	108 CFU/g	*Epinephelus coioides*	60 days (2x)	↑FBW, WG, SGR ↓FCR	[85]
HK *Leuconostoc mesenteroides*	107 CFU/g	*C. gariepinus*	60 days (2x)	↑FBW, WG, SGR, PER ↓FCR	[86]
HK *Edwardsiella sp*					

2 ↑: higher result compared to control; ↓: lower result compared to control; FBW: final body weight; WG: weight gain; ADG: average
daily gain; SGR: specific growth rate; RGR: relative growth rate; BMI: body mass index; FI: feed intake; FR: feeding rate; FE: feed
19 efficiency; FCR: feed conversion ratio; FCE: feed conversion efficiency; DE: digestive enzyme; PER: protein efficiency ratio; PPV
3 protein protective value; PR: Protein retention; LR: lipid retention; IGF-1: Insulin-like Growth Factor 1; IGF-2: Insulin-like Growth
4 Factor 2; GH1: Growth Hormone 1; GHr: Growth Hormone Receptor; GK: Glucokinase; HK1: Hexokinase 1; G6pase: Glucose-6-
Phosphatase; PK-L : Pyruvate Kinase, Liver Lysozyme

Overall, Table 2 highlights the beneficial effects of various biotic supplement to support the growth performance of aquactic organisms. Among these, probiotics, particularly *Bacillus* and *Lactobacillus* strain remain the most frequently studied and widely applied. However, when comparing biotic types, the effectiveness of prebiotics, synbiotics, postbiotics, and parabiotics is also evident, with all demonstrating consistent improvements in key growth parameters, including FBW, WG, SGR. The use of postbiotics, especially cell-free supernatants, offers a practical solution to the limitations associated with maintaining live bacterial viability, though the precise composition of these metabolites still requires further characterization. Meanwhile, parabiotics, primarily produced through heat inactivation, have shown promising results in promoting growth without the risks of introducing live microorganisms.

5.2 Enhance reproduction

Biotic supplements become a promising approach to enhance the reproduction in organisms, as they have the potential to improve fertility, fertilization rates, and hatching rate (Table 3). This effect is largely mediated through the regulation of sex hormones and genetic pathways associated with reproduction, mainly via hypothalamus–pituitary axis. The primary mechanism involves the enhancement of egg and sperm quality and quantity, as well as an increase in fecundity rates [87]. These biotic supplements may influence the expression of genes related to hormone production, gonadal development, and oogenesis or spermatogenesis, thereby improving reproductive outcomes in both males and females [88]. Several studies have found that the addition of biotic supplements in aquatic animal have a positive effect on reproduction. Gioacchini *et al.* [89] reported that the addition of *Lactobacillus rhamnosus* IMC50 at 10^6 CFU/g increase luteinizing hormone receptor (l hr) levels and a decrease in
29 transforming growth factor beta 1 (tgfb1) and growth differentiation factor 9 (gdf9) in female adult zebrafish (*Danio rerio*) which help in oocyte maturation. A mature oocyte will increase the success of the fertilization process by ensuring proper meiotic progression, cytoplasmic competency, and synchronization with spawning, ultimately
14 leading to improved embryonic development and higher hatchability rates. A similar result was also obtained in a study conducted by Qin *et al*. [90]. They found that the addition of *Lactobacillus casei* and *Lactobacillus rhamnosus* at 10^8 CFU/g can increase the fertility of 7-month-old *Danio rerio* and enhanced fish's fecundity through the increase expression of leptin, kisspeptin2 (kiss2), gonadotropin releasing hormone 3 (gnrh3), and luteinizing hormone (lh),

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luteinizing hormone/choriogonadotropin receptor (lhcgr), progestin and adipoQ receptor family member 8 (paqr8) gene. These genes are responsible for encoding neuropeptide hormone and oocyte maturation, respectively. Simlarly, the addition of prebiotics, particularly chitosan has been shown to influence reproductive development by enhancing gene expression and reproductive cell activity. This occurs through improved gonad development and stimulation of reproductive hormone secretion by hypotalamus. Interestingly, during the stimulation of hypotalamus to secrete reproductive hormone (gonadthropic hormones) by the pituary glands, it also secretes dopamine that can halt the gonad development. The supplementation of chitosan can act as dopamine antagonist and therby allowing continued gonad development [91].

Table 3: The effect of potential biotic supplements on animal reproduction

Biotic Supplements	Dose	Host	Durations	Results	Ref
Probiotics					
Bacillus subtilis, *Bacillus licheniformis*	0.03 g/m^3	Male *Oreochromis niloticus x Oreochromis mossambicus*	20 days	↑ T, GSI, HSI, VSI	[92]
		Female *O. niloticus x O. mossambicus*		↑ LH, FSH, P4, E2, GSI, HSI	
Bacillus spp.	0.0013 g/L (1.5 x 10^{10} CFU)	Female *O. niloticus*	60 days (1x a week)	↑ AF, RF, FSH, LH ↑= ED, P4	[93]
		Male *O. niloticus*		↑ LH, E ↑= FSH	
Bacillus cereus NP5	10^6, 10^8 CFU/g	Female *Clarias gariepinus*	84 days (2x)	↑ AF, HR, LS	[94]
Lactobacillus rhamnosus (IMC 501®)	10^6 CFU/ml	*Fundulus heteroclitus*	8 days	↑ AF, GSI,	[95]
Pediococcus acidilactici (Bactocell®)	10^8 CFU/g	*Carassius auratus*	180 days	↑ SM, SD, ED, OD, AF, RF, GSI, HSI, FR, HR	[96]
Bacillus sp. NP5	10^8 CFU/g	Male *C. gariepinus*	60 days (2x)	↑ SpC, SD, SM, SV	[97]
L. rhamnosus GG	10^8 CFU/g	MCLR induced female *Danio rerio*	28 days (2x)	↑ FR, HR, CEC, E2, T, VTG	[98]
B. subtilis, B. cereus	0.6 g/kg (4 x 10^{11} CFU/g)	Male *Rhamdia quelen*	90 days (4x)	↑ GSI, HSI, VSI, SCS	[99]
		Female *R. quelen*		↑ FR, VSI, DVO	
Prebiotics					
Yucca extract (AQUA-YUCCA)	0.0013 g/L	Female *O. niloticus*	60 days (1x a week)	↑ AF, ED, FSH, LH, E, P4, T ↑= RF	[93]
		Male *O. niloticus*		↑FSH, LH, E, P4, T	

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Biotic Supplements	Dose	Host	Durations	Results	Ref
MOS	1.5 g/kg	Female *O. niloticus x O. mossambicus*	40 days (2x)	↑FSH, LH, E2, P4, GSI, HSI, VSI, ED	[100]
		Male *O. niloticus x O. mossambicus*		↑T, GSI, HSI, VSI	
Chitosan	1 g/kg	*Barbonymus gonionotus*	60 days (2x)	↑FSH, LH, HR, FR, GSI, RF ↓ OP	[91]
Synbiotics					
Yucca extract (AQUA-YUCCA) + *B. subtilis, B. licheniformis, L. acidophilus* (UNI ECOTREAT)	0.0013 g/L	Female *O. niloticus*	60 days (1x a week)	↑ AF, RF, FSH, LH, E, P4, T ↑= ED	[93]
		Male *O. niloticus*		↑FSH, LH, E, P4, T	
acidophilus, B. longum, B. thermophylus, Streptococcus faecium, live yeast, oligosaccharides, amylase, protease, cellulose, pectinase, xylanase, phytase (Hydroyeast Aquaculture®)	15 g/kg	Male *O. niloticus*	56 days (2x)	↑ TW, GSI, SC, SM ↓ SA, DS	[101]
	10 g/kg	Female *O. niloticus*		↑ OW, ED, AF, RF	

↑: higher result compared to control; ↓: lower result compared to control; SM: sperm motility; SD: sperm density; SV: sperm volume; sV: semen volume; SpC: spermatocrit; SCS: sperm cell survival; SC: sperm count; SA: sperm abnormalities; DS: dead sperm; TW: testes weight; ED: egg diameter; OD: ovum diameter; OW: ovarium weight; CEC: cumulative egg count; OP: ovulation period; AF: absolute fecundity; RF: relative fecundity; WF: working fecundity; FR fertilization rate; HR hatching rate; LS: larvae survival; GSI gonadosomatic index; HSI hepatosomatic index; VSI: viscerosomatic index; E2: estradiol; E: estrogen; VTG: vitellogenin; T: testosterone ; LH: luteinizing hormone; FSH: follicle-stimulating hormone; DVO: diameter of vitellogenic oocytes; P4: progesterone

Table 3 shows the potential of probiotics, prebiotics, and synbiotics to improve reproductive performance in aquaculture organisms, primarily through the upregulation of reproductive hormones and the development of reproductive cells. However, currently, the application of postbiotics and parabiotics in this context remais unexplored. Given their higher stability and safety profile compared to probiotics, it is interesting to see the potential effect of those biotics alongside commonly used biotics supplements. Additionally, the current body of research is heavily focused on vertebrate fish species, with a no studies on commercially important invertebrates, such as shrimp.

5.3 Boost innate and adaptive immune system and protect against pathogen

Biotic supplements become a promising safer alternative to prevent aquatic diseases (Table 4). The overall mechanism on how probiotics and its derivative able to inhibit pathogenic bacteria in aquaculture are due to their metabolites that have bactericidal or bacteriostatic effect, improving host gut barrier, nutrient or adhesion competition with pathogenic bacteria, and modulation of host immune system [102]. The increase of antioxidant enzyme activity and immune related genes can increase an organism's survival rate when challenged with aquatic

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pathogenic bacteria [102, 103]. Chen *et al.* [104] showed that oral administration of *Paenibacillus ehimensi* isolated from Nile tilapia culture pools with the concentration of 10^7 CFU/g to Nile tilapia able to increase their survival rate infected with *Aeromonas hydrophila*. Analyzed by non-specific immune system parameters, the probiotics enhanced TNF-α and IL-β expression. Another strain of probiotics also have been tested on Nile Tilapia, such as *Bacillus velezensis*, *Lactobacillus plantarum*, *Enterococcus faecium* [61, 105]. Additionally, a study using synbiotic composed of MOS or XOS with *Pediococcus acidilactici* or *E. faecalis* showed an increase in lysozyme activity in narrow-clawed crayfish (*Astacus leptodactylus*). A significant increase of survival rate was also found after crayfish infected with *Aeromonas hydrophila* compared to control and MOS or XOS only [78]. Similar results also found using heat-killed *L. plantarum* in Red tilapia at the concentration of 4 mg/kg able to increase lysozyme content compared to control [106]. This bacteria strain is also used for *Claria macrocephalus* and showed similar results [107].

Table 4: The effect of potential biotic supplement on aquatic organism innate, adaptive immune system and blood profile

Biotic Supplements	Dose	Host	Pathogen	Dose	Infection Model	Results	Ref
Probiotics							
Bacillus velezensis, *Lactobacillus plantarum* N11	10^7; 10^8 CFU/g	*Oreochromis niloticus*	*Streptococcus agalactiae*	10^7 CFU/ml	IP injection (0.1 ml) for 15 days	↑ LYZ, RBA, PA (before challenge) ↑ SR	[60]
Encapsulated *Pediococcus acidilactici* (Bactocell PA10)	0.1, 0.2, 0.3 g/m^3	*O. niloticus* fingerlings	*Aspergillus flavus*	4 x10^3 conidia/ml	injection (0.2 ml) for 15 days fed with control diet	↑ RBC, Hb, WBC, P, CAT, GPx, SOD, LYZ, IgM, TNF-α, IL-1β, IL-8 (before challenge) ↓MDA ↑ SR	[108]
Paenibacillus ehimensis NPUST1	10^7 CFU/g	*niloticus*	*Streptococcus iniae*	10^5 CFU/ml	IP injection (0.02 ml) for 7 days fed with control diet	↑ PHG, LYZ, RB SOD ↑ TNF-α, IL-1β ↑ SR	[103]
			Aeromonas hydrophila	10^6 CFU/ml			
Bacillus subtilis L10	10^8 CFU/ml	*Litopenaeus vannamei*	*Vibrio harveyi*	10^6 CFU	third abdominal segment injection	↑ProPo, PE, LGBP, SP ↑ SR	[109]
B. subtilis G1							
B. subtilis, *Bacillus licheniformis* (Sanolife PRO-W ®)	0.03 g/m^3 (5 x 10^5 CFU/g)	*L. vannamei*	*Fusarium solani*	5 × 10^4 conidia/ml	swab on wounded section for 14 days	↑SOD, CAT, GPx, ProPO, LYZ, RB, Phag ↑ SR ↓ MDA	[110]
L. plantarum Ep-M17	5 x 10^8 CFU/g	*L. vannamei*	*Vibrio parahaemolyticus E1*	10^7 CFU/ml	immersion for 2 days	↑ SOD, AKP, CAT ↑ SR	[111]

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Biotic Supplements	Dose	Host	Pathogen	Dose	Infection Model	Results	Ref
Bacillus cereus	10^6 CFU/g	*Carassius auratus* var. Pengze	-	-	-	↑ TSOD, CAT, GSH, GPx, TLR2, IL-10 ↓ MDA, TNF-α, IL-1β, IFN-Ɣ	[112]
Bacillus amyloliquefaciens	10^5, 10^7, 10^9 CFU/g	*Labeo rohita*	*hydrophila*	10^7 CFU/ml	IP injection (0.1 ml) for 28 days	↑ SOD, CAT, LYZ, IgM ↓ MDA ↑ SR, AST, ALT	[113]
B. velezensis	10^6 CFU/g	*Dicentrarchus labrax*	*Vibrio anguillarum*	2×10^6 CFU/ml	IP injection (0.1 ml) for 6 days	↑ LYZ, NO, Phag, IL-1β, TNF-α, COX-2, DIC ↑ SR	[114]
Prebiotics							
MOS	6 g/kg	*Pangasianodon gigas* × *P. hypophthalmus*	*Edwardsiella ictaluri*	6 x 10^7 CFU/ml	IP injection (0.1 ml) for 2 weeks	↑ WBC, Hb, LYM, Ig, LYZ (before challenge) ↑ SR	[74]
AXOS + inulin	4 mg/g each	*L. vannamei*	*Vibrio alginolyticus* WSSV	2 x 10^7 CFU/ml	ventral sinus of cephalothorax injection (0.2 ml) for 5 days	↑ PO, MsyD88, ERK ↑ SR	[75]
GOS	5 g/kg	*Acipenser baerii* × *A. schrenckii*	*hydrophila*	1.5 x 10^6 CFU/ml	injection (0.2 ml) for 2 weeks	↑ACP, AKP, LYZ, MPO, SOD, CAT, GPx, ↓ MDA ↑ SR	[76]
Chitosan	1 g/kg	*Barbonymus gonionotus*	-	-	-	↑ IgM, LYZ,	[91]
GOS	20 g/kg	*Astacus leptodactylus Eschscholtz, 1823*	-	-	air stress	↑ PO, LYZ, SOD	[115]
FOS, β-glucan	0.5, 1, 1.5 g/kg	*O. niloticus*	*Fusarium oxysporum*	4 x10^3 conidia/ml	injection for 15 days	↑ SOD, CAT, GPx, LYZ, IgM ↓ MDA ↑ SR	[116]
β-glucan	0.5, 1, 2 g/kg	*Oncorhynchus mykiss* fingerlings	-	-	-	↑ IgM, LYZ, C3, C4, ACH50.	[117]
Synbiotics							

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Biotic Supplements	Dose	Host	Pathogen	Dose	Infection Model	Results	Ref
Corn Cob derived XOS + *L. plantarum* CR1T5	10 g/kg; 10^8 CFU/g	*O. Niloticus*	*agalactiae*	10^7 CFU/ml	IP injection (0.1 ml) for 15 days	↑ skin mucus LYZ, POD (before challenge) ↑serum LYZ, POD, ACH50, Phag, RB (before challenge) ↑ SR	[77]
GOS + *Enterococcus faecalis*	10 g/kg; 6.9 x 10^7 CFU/g	*A. leptodactylus*	*hydrophila*	10^8 CFU/ml	fifth thoracic legs injection (20 ml) for 2 days	↑SOD, LYZ, NO, PO (before challenge) ↑ SR	[78]
MOS + *E. faecalis*							
B. subtilis, S. cerevisiae, yeast cell wall, *B. subtilis* fermentation, yucca extract (Bioture)	3 g/kg	*L. vannamei*	*V. parahaemolyticus*	1.75 x 10^6 CFU/ml	injection (0.025 ml) for 2 days	↑ TSOD, LYZ, CAT, ACP, ALT ↓ MDA ↑ SR	[118]
Postbiotics							
Bacillus, Lactobacillus, Saccharomyces cell-lysate	20 g/kg	*Macrobrachium nipponense*	-	-	-	↑ POD, PO, LYZ, AKP, MAPK7, MAK14, IRAK4 ↓ MDA	[85]
L. plantarum VAL6 EPS	4 g/kg	*L. vannamei*	*V. parahae-molyticus*	10^5 CFU/ml	immersion for 9 days	↑ PO, RB, SOD	[119]
Bifidobacterium bifidum VAR2 EPS							
Staphylococcus lentus bacteriocin BS-SLSZ2	18 mg/ml	*Artemia salina*	*V. harveyi*	10^6 CFU	immersion for 7 days	↑ SR	[120]
			aeruginosa				
Clostridium butyricum metabolites		*Ctenopharyngodon idella*	-	-	high cottonseed and rapeseed diet	↑ SOD, CAT, GSH = C3, IgM, ACP ↓ MDA	[121]
Parabiotics							
HK *B. pumilus* SE5	10^8 CFU/g	*Epinephelus coioides*	-	-	-	↑ Phag, SOD, IgM, C3, IL-8, IL-1β, TLR-2	[85]

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Biotic Supplements	Dose	Host	Pathogen	Dose	Infection Model	Results	Ref
HK *Pseudoalteromonas piscicida* 2515	10^5 CFU/ml	*Paralichthys olivaceus*	*V. anguillarum*	2.5 x 10^7 CFU/ml	immersion for 10 days	↑ SOD, CAT, POD ↑ IL-10, TNF-α (spleen) ↑ LYZ, IL-1β, TNF-α (head-kidney) ↑ SR	[122]
HK *L. plantarum* (House Wellness Foods Corp)	50 ppm	*P. hypophthalmus*	*Edwardsiella ictaluri*	10^5 CFU/ml	IP injection 0.1 ml) for 14 days	↑ LYZ, WBC, RBC ↑ SR	[123]
HK *P. aeruginosa* VSG2	30 mg/kg	*Cyprinus carpio*	*hydrophila*	10^7 CFU/ml	IP injection (0.1 ml) for 15 days	↑ mucus and serum LYZ, Ig, ALP, SOD, CAT, GPx, GSH, ↑ SR ↓ MDA	[124]
HK *B. subtilis*	10^8 CFU/g	*P. hypophthalmus*	-	-	-	↑ Phag, SOD, CAT, GPx ↓ MDA	[125]
Formalin-killed *B. amyloliquefaciens* COFCAU_P1	10^8 CFU/g	*L. rohita* fingerlings	*hydrophila*	$10^{4.5}$ CFU/ml	IP injection (0.1 ml) for 14 days	↑ SOD, GPx ↑ SR	[126]

↑: higher result compared to control; ↓: lower result compared to control; SR: survival rate; RBC: red blood cells; Hb: haemoglobin; WBC: white blood cells; Phag: phagocytosis; RB: respiratory burst; GLB: globulin; IgM: immunoglobulin M; LYZ: lysozyme; CAT: catalase; GPx: glutathione peroxidase; SOD: superoxide dismutase; POD: peroxidase; TSOD: total superoxide dismutase; GSH: glutathione; NO: nitric oxide; ProPo: prophenoloxidase; MDA: malondialdehyde; PE: peroxinectin; LGBP: lipopolysaccharide and β-1,3-glucan-binding protein; SP: serine protein; CR: crustin; lvLEC: lymphatic vessel endothelial cells; PEN-3α: PEN-3 alpha; COX-2: Cyclooxygenase-2; DIC: dicentracin; ACH50: alternative complement activity; Myd88: myeloid differentiation primary response gene 88; ERK: extracellular signal-regulated kinase; C3: complement component 3; C4: complement component 4; MAPK7: mitogen-activated protein kinase 7; MAK14: mitogen-activated protein kinase-activated protein kinase 14; IRAK4: interleukin-1 receptor-associated kinase 4; TLR-2: toll-like receptor 2; TNF-α: tumor necrosis factor-alpha; IL-1β: interleukin-1 beta; IL-8: interleukin-8; IL-10: interleukin-10; IFN-ɣ: interferon gamma; AKP: alkaline phosphatase; ALT: alanine aminotransferase; ACP: acid phosphatase

Table 4 shows how biotic supplements can help protect aquaculture species from common bacterial infections during the rearing process. In many studies, fish or shrimp are intentionally exposed to harmful bacteria, usually through injection or immersion to test how well the biotics work. After exposure, the animals are either kept on a supplemented diet or returned to a regular one. The disease protection conferred by biotic supplements is closely linked to their ability to enhance both the innate and adaptive immune responses of the host. Specifically, biotics have been shown to upregulate anti-inflammatory cytokines while downregulating pro-inflammatory cytokines, thereby improving the host's resistance to infection, mortality, and overall immune regulation.

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5.4 Improve Water Quality

The water quality is commonly checked on several parameters, including temperature, pH, DO, nitrite, nitrate, ammonium, and salinity. To obtain water quality, rapid analysis method usually employed by using a colorimetric test-kit [127]. Maintaining water quality is a vital step in aquaculture production. For example, a study conducted by Evans *et al*. [128] found that Nile Tilapia exposed to sublethal DO levels resulted in higher stress determined via blood glucose and lower their survivability against *Streptococcus agalactiae*. Other than that, Abbink *et al*. [129] studied the effect of different pH and temperature on juvenile yellowtail kingfish (*Seriola lalandi*) in recirculating aquaculture systems (RAS) and found that the pH of 6.58 and temperature of 21°C resulted in the lowest final weight.

Accumulation of aquatic animal waste also poses a danger in aquaculture environments, unmanaged waste can result in high ammonia content, the final product of protein metabolism. High ammonia content is toxic to the animals, thus required to be broken down into other substances. Several studies found that the incorporation of probiotics in aquaculture can help to improve water quality parameters (Table 2). A study by Thurlow *et al*. [63] using *Bacillius velezensis* AP193 during the production of catfish (*Ictalurus punctatus*) can reduce the amount of phosphorus, nitrogen, and nitrate-nitrogen from 0.136 ± 0.010, 0.344 ± 0.051, 0.051 ± 0.019 to 0.110 ± 0.013, 0.195 ± 0.025, 0.013 ± 0.005, respectively. Another study found that using *Pediococcus acidilactici* in Nile tilapia (*Oreochromis niloticus*) farming can reduce the total ammonia nitrogen (TAN) and ammonia (NH_3) proportional to the amount of *P. acidilactici* added, with the lowest concentration TAN and NH_3 at 0.43 and 0.04 ppm [108]. A bacteria consortium containing *Bacillus cereus*, *Bacillus amyloliquefaciens*, and *Pseudomonas stutzeri* can become an ammonia-oxidizing and nitrite-oxidizing agent. These bacteria can reduce the ammonia, nitrite, and nitrate content. However, the activity of nitrite oxidation is slow; this may happen because two out of three bacteria in the consortium are ammonia oxidizing agents [130]. Prebiotics like MOS have shown promising potential to improve water quality in aquaculture systems. For example, in a study by Kishawy *et al*. [131] with Nile tilapia raised in a biofloc setup, MOS supplementation helped reduce harmful compounds such as, TAN, nitrite, and nitrate by around 50% compared to the control group. This effect was seen regardless of whether the fish were fed plant-based or fish-based diets. This study also compared MOS with glycerol as carbon source, both shows similar result for the water quality. However, MOS also found to increase the growth of beneficial microbes, particularly *Bacillus* species, in the biofloc. This suggests that MOS can help support the natural growth of native bacteria in the system.

In another study by Saleh *et al*. [93], a plant-based prebiotic, *Yucca schidigera,* combined with commercial probiotics (*Bacillus subtilis*, *Bacillus licheniformis*, and *Lactobacillus acidophilus*), significantly reduced TAN from 1.25 mg/L to 0.9 mg/L, and total suspended solids (TSS) from 136 mg/L to 73 mg/L. However, ammonia oxidation led to a slight increase in nitrate concentration, from 0.136 mg/L to 0.192 mg/L, indicating the potential need for denitrifying bacteria to further convert nitrate into nitrogen gas. The study also found that synbiotics was more effective than using either probiotics or prebiotics alone.

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Table 5: The effect of potential biotic supplements on aquaculture water quality

Biotic Supplements	Dose	Host	Durations (each day)	Results	Ref
Probiotics					
Bacillus subtilis powder	4.76 $\times 10^8$ CFU/g	*Oreochromis niloticus*	50 days (3x)	↓TAN, NO_2, NO_3	[132]
Pediococcus acidilactici	10^6 CFU/ml	*Litopenaeus vannamei*	110 days	↓NH_4^+, $NH3_3$ NO_3	[133]
Bacillus cereus				↓NH_4^+, NO_3 NO_2 =NH_3	
Bacillus toyonensis	1 or 2 × 10^5 CFU/ml	*O. niloticus*	70 days (3x)	=NH_3	[134]
Geobacillus stearothermophilus					
B. subtilis, Lactobacillus sp., Saccharomyces sp. Entrobacterium spp. (Prozym powder Ultra ®)	0.002 g/m^3	*O. niloticus* fingerlings	56 days (1x)	↓NH_4^+, NO_3 NO_2	[135]
B. subtilis, B.cereus, S. cerevisiae (Microban aqua ®)	0.002 g/m^3				
B. subtilis, Paracoccus sp., P. acidilactici, Enterococcus sp., Thiobacillus sp. (Aquastar®)	0.0015 g/m^3				
B. subtilis, Bacillus licheniformis, Bacillus pumilus (Sanolife PRO-W ®)	0.0010 g/m^3				
B. subtilis, *B. licheniformis*	0.01, 0.02, 0.03 g/m^3	Male and female *O. niloticus* × *O. mossambicus*	20 days	↓TAN, NH_3	[92]
Prebiotics					
MOS	Vary, maintaining the C:N ratio of 15:1	*O. niloticus*	50 days (3x)	↓TAN, NO_2, NO_3	[131]
Synbiotics					
de-potash vinasse (DPV) + *Enterobacter ludwigii* HS1-SOB, *Pseudomonas stutzeri* B6-SOB, *Cytobacillus firmus* C8-SOB	1 L mixed ith sand/1.5 ha pond	*L. vannamei*	80 days	↓NH_3, PO_4^{3-}	[136]
Yucca extract (AQUA-YUCCA) + *B. subtilis, B. licheniformis, L. acidophilus* (UNI ECOTREAT)	0.0013 g/L	*O. niloticus*	60 days (1x a week)	↑ NO_3 ↓TAN	[93]

TAN: total ammonia nitrogen, NO_2: Nitrite, NO_3: Nitrate, NH_3: Ammonia, NH_4^+: Ammonium, PO_4^{3-}: Phosphate

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The application of postbiotics and parabiotics for improving water quality remains unexplored. However, there is a study using feed added with pepsin resulted in lower ammonia, nitrate, and nitrite content in the water. This effect is attributed to pepsin's ability to hydrolyze dietary proteins into simpler compounds, enhancing protein digestibility and thereby decreasing the excretion of nitrogenous waste into the aquatic environment [137]. This founding can be interesting to look for, because microogranism have the abilities to produces specific enzymes, including pepsin-like proteases that can aid in protein hydrolysis [138].

5.5 Comparative analysis of biotic supplements in aquaculture settings

The biotic application in aquaculture offers numerous benefits, contributing to more sustainable and efficient aquaculture production. Among the various types, probitocis remain the most widely studied and applied. In contrast to newer biotic supplements that are still emerging in the field. The application of these biotics supplement is vary, but the most common are done through feed mix or directly mix it to the water. Probiotics have been applied in a wide range of dosages, with beneficial effects observed even at lower concentrations, although some studies use up to 10^{10} CFU/g. The feeding duration in probiotic studies ranges from 14 to 180 days. However, it is best to introduce probiotics at earlier stage of life. This is likely because probiotics can more effectively colonize the gut before a stable and established gut microflora is formed, in both shrimp and fish species [139,140]. With that being said, probiotics as a living microorganism have the abilities to acquire resistance gene [20, 141] and require a appropriate storage condition to maintain its viability. Compared to probiotics, prebiotics offer a more practical and potentially easier approach to modulating the gut microbiota in aquatic animals. Prebiotics work by promoting the growth of beneficial microbes already present in the host's digestive system or environemnts [142]. However, their effectiveness is highly dependent on the native microbial composition, which varies between those two. This variability requires careful analysis of the host's gut microbiota to ensure the prebiotic used is appropriate and effective. Commonly used prebiotics in aquaculture include GOS, FOS, XOS, β-glucan, and chitosan, although novel plant-based prebiotics are also being explored. These compounds are typically added in relatively low amounts, around 0.05% to 5% per kg of basal diet, and are administered over feeding periods similar to those used for probiotics.

Synbiotics can help extend the viability and effectiveness of probiotics by providing a prebiotic that supports their growth. For instance, a study by Huynh *et al*. [142] found that adding 2% white sweet potato extract enhanced the growth of *Lactobacillus* spp. However, the same extract also promoted the growth of pathogenic bacteria such as *Vibrio harveyi* and *Vibrio parahaemolyticus*, as reflected by a low prebiotic score. This highlights the importance of carefully selecting prebiotics that specifically support beneficial bacteria without aiding harmful ones. In addition to supporting growth, synbiotics can also improve the colonization of probiotics in the host's gut [141]. Boonanuntanasarn *et al*. [75], for example, reported that shrimp fed a combination of β-glucan and *Bacillus subtilis* showed higher levels of lactic acid bacteria (LAB) and reduced Vibrio counts in the intestine after 90 days of feeding. However incorporating synbiotics in aquaculture can become more costly, especially when 60–80% of operational cost is accounted for feed [141]. Although growth improvement increases overall productivity, it still can be challenging for small-scale farmer that is found many in Indonesian aquaculture [145].

Postbiotics and parabiotics are emerging as promising biotic components to support more sustainable aquaculture practices. They are considered safer and more convenient alternatives to probiotics and synbiotics, as they can deliver similar benefits without the risks associated with live microorganisms. Compared to other biotics, research on postbiotics and parabiotics is still limited, particularly in understanding their modes of action [146]. Therefore, laboratory-scale feeding trials are essential before implementing these biotics in real-world aquaculture

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systems. Additionally, since the composition and concentration of active metabolites can vary depending on microbial growth conditions, metabolomic studies are crucial. These studies help identify and quantify bioactive compounds using advanced analytical techniques such as mass spectrometry (MS), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), or nuclear magnetic resonance (NMR) [147].

6. Biotic Supplementation in aquaculture of Indonesia: Current state

In Indonesia, the use of biotic supplements in aquaculture has gained attention as the industry seeks practices that are sustainable and efficient. They are being integrated into the farming of various aquatic species, mostly in shrimp and fish, to enhance growth rates, improve feed conversion ratios, and enhance disease resistance from aquatic pathogenic bacteria. Table 6 summarizes several studies that utilizevarious biotics supplement. Biotic supplements have shown to be effective in combating common diseases in Indonesian aquaculture, such as white spot syndrome virus (WSSV) in shrimp [148] and bacterial infections caused by Aeromonas in freshwater fish [147, 148, 149] which both are a devastating reason for economic losses for aquaculture farmer.

Table 6: Current status of studies in aquacultural biotic supplements in Indonesia

Biotic Supplements	Host	Pathogen	Result	Ref
Probiotics				
Bacillus megaterium PTB 1.4 *Pediococcus pentosaceis E2211*	*Clarias sp.*	*Aeromonas hydrophila*	The supplementation of both *B. megaterium* PTB 1.4 *P. pentosaceus* E2211 can increase catfish survival rate and immune response.	[149]
Bacillus NP5	*Clarias gariepinus*	*A. hydrophila*	The supplementation of *Bacillus* NP5 at 10^9 CFU/ml can increase fish growth, survival rate, and immune response.	[150]
Bacillus subtilis, Lactobacillus casei	*C. gariepinus*	*A. hydrophila*	The supplementation of *B. subtilis* and *L. casei* can increase catfish survival rate and immune response.	[151]
B. subtilis	*Epinephelus fuscoguttatus*	-	The supplementation of *B. subtilis* significantly affect protein digestibility efficiency of diet utilization, growth, survival rate, and enzyme activities.	[152]
Synbiotic				
Pseudoaltermonas piscicida 1Ub + MOS	*Litopenaeus vannamei*	*Vibrio harveyi*	Microencapsulated *P. piscicida* 1Ub enriched with MOS through *Artemia* sp. increase the survival rate and immune response after *V. harveyi* infection.	[153]

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Biotic Supplements	Host	Pathogen	Result	Ref
Bacillus NP5 + honey	*L. vannamei*	*Vibrio parahaemolyticus*	The supplementation of *Bacillus* NP5 and honey can increase the shrimps' growth performance and immune response.	[154]
Postbiotic				
Nodulisporium sp. KT29 metabolites	*L. vannamei*	*V. harveyi* WSSV	The supplementation of 20 mg/kg feed of *Nodulisporium* sp. KT29 can increase the phenoloxidase activity and respiratory burst and increase survival rate.	[148]
Bacillus cereus SN7 and *Vagococcus fluvialis* CT21 culture supernatant	-	*Vibrio alginolyticus* *A. hydrophila* *Pseudomonas aeroginosa*	Both culture supernatant shows antimicrobial activity to three fish pathogen using disc diffusion method.	[155]
Parabiotic				
HK *Lactobacillus plantarum*	Juvenile *Oreochromis* sp.	*Streptococcus agalactiae*	The supplementation of HK *L. plantarum* at the concentration of 10-20 mg/kg feed can increase tilapia growth and the concentration of 250 mg/kg offers higher survival rate.	[156]
HK *Bacillus* NP5	*Oreochromis niloticus*	*S. agalactiae*	The supplementation of HK *Bacillus* NP5 at 10^{10} CFU/ml can increase the average final weight and specific growth rate, increase enzyme activity (lipase, protease, and amylase). The paraprobiotics also induced immune response	[157]

Indonesia has been actively conducting research on the use of various biotic supplements to enhance aquaculture production. This growing body of work suggests that Indonesia is taking important steps toward strengthening its aquaculture sector, in line with the national goal of positioning aquaculture as a competitive and sustainable pillar of economic growth, and the mission to develop a responsible and environmentally friendly aquaculture industry [145]. However, many challenges remain particularly for small-scale farmers. These challenges can be grouped into internal and external factors. Internal challenges include issues like poor feed and egg quality, low water quality, and limited capacity to manage aquatic diseases and parasites. On the other hand, external challenges involve limited access to government support, lack of training, and insufficient profitability. These barriers often prevent small-scale farmers from achieving sustainable production goals [145]. In this context, the use of biotic supplements offers promising solutions by helping to improve feed utilization, enhance animal health, and reduce dependency on chemical treatments, making them a valuable tool in overcoming these challenges.

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7. Challenges and safety concerns

While biotic supplements offer numerous benefits in aquaculture, their use also comes with several challenges and safety concerns that must be carefully addressed. The effectiveness of these supplements heavily depends on factors such as species, dosage, and duration of treatment. Therefore, preliminary studies are essential when introducing a specific biotic to a new host species to assess its effectiveness and safety.

Among the most significant challenges associated with probiotics and synbiotics are maintaining microbial viability, ensuring successful colonization of the host's gut, and avoiding the risk of antibiotic resistance gene transfer, which may pose a threat to human health [141]. Although no adverse effects of probiotics have been reported in aquaculture to date, gastrointestinal side effects of probiotics have been reported in humans with inflammatory bowel disease, which can depend on the dose and probiotic strain [158]. This underscores the importance of confirming the safety of probiotics in aquaculture species, especially as they can persist in aquatic animals, which are often consumed raw or half-cooked by humans.

Synbiotics present an additional challenge in that their success depends on selecting the right combination of probiotic and prebiotic strains that work synergistically [18, 29]. On the other hand, prebiotics may be considered a safer option since they are non-living and do not carry the same risks as live microbes. However, their effectiveness relies on the presence of specific native microbiota, which can vary widely. In some cases, prebiotics may even support the growth of pathogenic bacteria, making microbial profiling of the host environment a critical step. Moreover, there is currently no standardized regulation for the use of prebiotics in aquaculture, leaving their application and outcomes largely uncontrolled [159].

Compared to these, postbiotics and parabiotics offer a potentially safer alternative, as they consist of either microbial metabolites or inactivated microbial cells, eliminating the risks associated with live organisms. However, their application is still in its early stages, and further research is needed to validate their efficacy. For postbiotics, metabolomic analysis is a crucial component, enabling the identification and quantification of bioactive compounds produced by microbes. Depending on the research goal, either targeted metabolomics or untargeted metabolomics [147, 159]. Targeted metabolomics focuses on analyzing and measuring specific, well-characterized metabolites of interest, while untargeted metabolomics aims to detect and profile all metabolites present in a sample, including unknown or unexpected compounds [147, 159]. The main challenge here lies in the high cost of equipment and the need for trained personnel, especially when using advanced tools like NMR [147]. For parabiotics, the key issue is determining the optimal inactivation method that preserves biological activity while ensuring safety [160]. It is also important to standardize and promote the concepts of postbiotics and parabiotics, particularly among farmers, to avoid confusion regarding the types of biotic compounds being used in aquaculture. Familiarizing farmers with these terms will help ensure proper application and improve acceptance of these emerging technologies in the field.

Currently, commercial products based on postbiotics and parabiotics remain limited, especially when compared to the more widely available probiotic, prebiotic, and synbiotic formulations. Nonetheless, ensuring the safe use of biotics in aquaculture is critical, not only for maintaining animal health but also for protecting human health, due to the direct connection between aquaculture products and the food chain.

8. Concluding remarks and future prospects

This review highlights the potential use of various biotics supplements as growth promotor, diseases protection, reproduction enhancement, and water quality improvement in aquaculture setting. Aquaculture is faced with growing demand and therefore the industry is shifting toward intensification to increase aquatic production. However, this practice often led to diseases susceptibility and high environmental waste, compromising animal welfare and elevate

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mortality rate. In this context, biotic supplements become a promising alternative to antibiotics for improving aquaculture sustainability. Despite this potential, the efficacy of biotic treatment can vary due to factors in species differences, dosage, species, and treatment duration. Newer biotics supplements, such as postbiotics and parabiotics, are gaining attention for potential implementation in aquaculture. These biotics offer several benefits, including greater stability during storage and the absence of live microorganisms, which minimizes the risk of disrupting the aquatic microbiome. However, research about their functional metabolites and standardized method of inactivation to preserve their bioactivity is still lacking. Additionally, their modes of action not yet fully understood and need to be explored more. Furthermore, in practical aquaculture setting, these terms can be unfamiliar or ambigous for farmer and need to be introduced to ensure shared understanding. Despite these challenges, advancements in biotechnology and metabolomic research are targeting to create more effective and specific biotic formulations. Continued investment in research, education, and regulatory frameworks will be crucial to fully harness the benefits of biotic supplements, transforming aquaculture into a more resilient and productive industry.

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