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## Probiotics and their application in aquaculture for improving the growth and immunity of fish

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**Abstract.** *To increase the digestibility and digestibility of feed, stimulate the growth and development of animals, and increase nonspecific immunity, enzyme, probiotic, prebiotic and combined enzyme-probiotic preparations, as well as complex probiotic preparations enriched with phytocomponents are used.*

*Probiotics for artificially grown fish are beneficial bacteria and live microbiological additives that are traditionally added to agricultural aquaculture systems to maintain health, productivity and growth. Therefore, a promising direction in aquaculture is the use of feed with probiotic cultures. The use of probiotics is associated with solving various health problems, in particular, with increasing the efficiency of digestion and stimulating growth and development. Probiotics, having a positive effect on the host's body, contribute to the restoration of digestion, biological status, and immune response and increase the effectiveness of vaccination. The use of probiotics significantly reduces the cost of treating diseases in animals, increases productivity and improves product quality. In recent years, many studies have been conducted on the use of various probiotics in fishing. This review is aimed at generalizing the topic of the possible use of gram-positive bacteria as probiotics in a comprehensive study of the existing literature. With the expansion of ideas about the biological effectiveness of probiotics and the proof that the structural elements of cells and their metabolites are at least effective in some cases. As a result, various types of bacteria, including bacilli, lactic acid bacteria, and microbes, were used to improve the growth and immunity of various fish. However, this study conducted a review of the scientific literature showing that probiotics in aquaculture have a positive effect on the overall productivity of fish.*

**Keywords:** *probiotics, aquaculture, fish, bacterial strain, review, lactic acid bacteria, feed.*

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### Introduction

Fishing is one of the main sources of food for humanity. Aquaculture has undergone significant changes in the last decades under the influence of scientific and technological advances. This sector is growing at an unprecedented rate and supplies more than half of the world's fish for human consumption [1].

Recently, the role of probiotics in fish farming is growing. This is primarily due to diseases, which represent a major challenge for fish farming and a huge problem for further growth and expansion. Probiotics for artificially farmed fish are beneficial bacteria that are traditionally added to aquaculture farming systems to support their health, productivity and growth [2]. The widespread use of antibiotics for the prevention and control of bacterial diseases in fish farms has led to problems such as drug resistance, accumulation of antibiotics

in tissue and immunosuppression. Thus, probiotics as a prophylactic agent are progressively used in aquaculture [3]. In this regard, currently, various probiotics are widely used as means of maintaining and restoring the normal physiological state of animals, and the interest of scientists and practitioners in the use of microorganisms in agricultural production is increasing significantly. [4].

It is crucial to raise fish using environmentally friendly feed and competitive in price and taste. Fish require natural living conditions more than any other agricultural animals because they are sensitive even to significant fluctuations in the parameters of the aquatic environment (availability of oxygen, pH, nutrient and microflora). Therefore, the biological role of nutrient-balanced diets is now being supplemented by the functional importance of friendly microflora, whose deficiency must be compensated artificially [5].

In recent years, scholars have conducted a series of studies on the use of probiotics in fish farming. Thus, it is important to analyze these papers to provide useful information on scientific progress. This review will thus focus on summarizing published articles until 2020 that report gram-positive microorganisms applied as probiotics and their effect on productive performance in different fish.

### **The role of probiotics in aquaculture**

Recently, more importance is given to industrial methods of fish breeding with the use of various types of feed. However, natural food contains a wider range of biologically active components that are regulators of many metabolic processes. Consequently, in addition to the balance of essential nutrients in the food for the rearing of physiologically full-fledged young fish, biologically active substances are important, which include feed probiotics [6].

The term “probiotics” is used to describe a broad class of microorganisms with antagonistic activity against pathogenic microflora. Probiotics are usually referred to as preparations based on live microbial cultures used for the correction of microbial cenosis during the treatment and prevention of a wide range of diseases associated with dysbiotic conditions [7]. Probiotics also help in the post-stress adaptation by increasing the resistance of the microorganism to pathogens; they improve the digestive system through the additional production of enzymes in the digestive tract. Also, probiotics reduce the accumulation of organic contaminants and effectively maintain water quality [8]. Modern probiotics can easily satisfy the desire for sustainable aquaculture as they can enhance two key factors: growth efficiency and disease resistance. Benefits of such supplements include improved feed value, enzymatic contribution to digestion, inhibition of pathogens, antimutagenic and anticarcinogenic activity, and enhanced immune response [9].

The efficiency of aquaculture is largely determined by the quality and quantity of used feed. Reducing feed costs is one of the main economic factors that increase the profitability of fish farming [10]. Probiotics that are added to the feed have a significant impact on feed consumption per unit of fish growth because they contribute to their fuller assimilation, neutralization of mycotoxins coming with feed, displace pathogenic microflora, strengthen the general resistance of the fish organism [4].

In previous studies, the beneficial effects of the use of probiotics in fish feed have been reported. According to published papers, the majority of commercial probiotics contain mostly *Lactobacillus* and *Bacillus* sp. [11,12].

### **General mechanism of probiotic action**

An effective probiotic for fish farming, able to populate the intestines, and has a dual beneficial effect. Probiotics realize their positive effect on the macroorganism through a whole arsenal of mechanisms, not all of which have yet been fully deciphered. Among the well-studied ones are antagonistic activity against pathogenic and conditionally pathogenic microorganisms [13]. Secondly, probiotics have an adhesive activity to intestinal epithelial cells and can successfully compete with pathogenic and conditionally pathogenic microbes for adhesion sites on the intestinal wall and, as a consequence, for limiting nutrients, which eventually also leads to

inhibition of undesirable microflora growth [14]. The effects of probiotics on nutrient utilization, digestion and growth have been studied extensively [2].

### **The gram-positive microorganisms applied as probiotics affect the growth performance and immunity of fish.**

*Bacillus species.* The search for microorganisms that can be used as probiotics represents the basis for the development of probiotic preparations. The microbial organisms used as probiotics or tested as potential probiotics are given in Table 1. As a result of years of targeted screening, strains like *Bacillus subtilis* and *B. licheniformis*, *B. coagulans*, *B. firmicutes* (*Paenibacillus polymyxa*), *B. pumilus* have been selected for the development of corrective probiotics [12,15]. Some experimental strains of these bacteria can possess distinctly expressed antagonistic activity to a wide range of pathogenic and conditionally pathogenic microorganisms [16]. Bacilli are saprophytes and are major participants in the decomposition of protein substrates. Owing to their high activity, they are often antagonistic to other microorganisms. One of the important advantages of these bacteria is their spore-forming ability, which allows them to remain viable under unfavourable environmental conditions (including the moisture-heat treatment of raw materials and feed in the process of pelleting and extrusion) [17]. Spore resistance can be explained by various factors, such as the presence of thick protein layers of spores, reduced permeability of the spore core, or reduced water content in the spore core (Mingmongkolchai and Panbangred 2018).

*Bacillus* spp. are well known for their ability to produce extracellular enzymes. When used in aquaculture feed, these enzymes can improve digestibility and reduce the feed conversion ratio. Ultimately, improving the feed conversion ratio in aquaculture feeds is economically beneficial because the feed can be a major cost to the producer and, also helps improve water quality by reducing waste [19]. Studies show that they secrete biologically active substances in the intestine and produce various digestive enzymes. As a result, digestion improves, feed assimilation increases, average daily weight gain increases, and the growth of fish are stimulated. For instance, *Bacillus* spp. isolated from the pond of common carp stimulated productive performance [20].

*Bacillus subtilis* bacteria, which are present and proliferate in the intestinal lumen, produce metabolites that inhibit the growth of pathogenic and opportunistic microflora, such as *staphylococci*, *protozoa*, *shigella*, *escherichia*, *pseudomonads*, *Candida* fungi and other organisms [21]. *Bacillus subtilis* bacteria are not capable of forming colonies in the intestines of fish - once the preparation is discontinued, they are gradually eliminated from the organism, but maintenance of their high concentration in the digestive tract increases the survival rate and productivity of fish [22].

Numerous publications have shown that probiotics with *Bacillus subtilis* improve growth and stimulate the immune system [12,15,23]. For instance, Opiyo et al. (2009) revealed that *B. subtilis* led to significantly higher protein at 5 g kg<sup>-1</sup> ( $P < 0.05$ ).

In addition, it has been reported that *B. coagulans*, *B. licheniformis* and *B. firmicutes* (*Paenibacillus polymyxa*) ( $P < 0.05$ ) enhanced the resistance of fish fry against bacterial challenge [24].

*Lactic acid bacteria (LAB).* LAB in probiotic supplements normalize the intestinal microflora not only by suppressing pathogens, but also by increasing immunity; their activity products - immunoglobulins and lactoglobulins have antimicrobial, antiviral, and anticancer effects [25].

Lactobacilli produce large amounts of acetic, formic, lactic acids and hydrogen peroxide, which have high antiseptic, bactericidal and antioxidant properties (Teame et al. 2020). One mechanism for preventing colonization by pathogens in the intestine is competition for adhesion sites on the surface of the intestinal epithelium. Bacteria that grow slowly but attach to the intestinal wall can colonize the intestine, while non-adherent species are compensated by increasing their growth rate. Attachment provides the microorganism with resistance to the leaching of intestinal contents. It follows that if a probiotic strain can occupy adhesion sites on the intestinal wall, it takes root in the digestive tract, and vice versa [27].

The beneficial effects of Lactobacilli of normal gut microflora have led to their widespread

use in probiotics. The following *Lactobacillus* species are most commonly used as probiotics in aquaculture: *L. acidophilus* [28], *L. helveticus* [11], *L. rhamnosus* [29,30], *L. plantarum* [31 - 33], *L. delbrueckii subsp. Lactis* [34, 35], *L. delbrueckii subsp. Bulgaricus* [36].

Ahire et al. (2019) reported that *Lactobacillus helveticus* showed antimicrobial activity against fish *Aeromonas* spp. In addition, it increased antioxidant levels and enhanced the selective absorption of essential trace elements in goldfish [11].

Furthermore, Soltani et al. (2019) evaluated the effects of probiotic supplementation on immune and growth performance in fish. The authors demonstrated that supplementation with *Lactobacillus plantarum* 426951 could improve the growth and some immune status of Rainbow trout vaccinated against yersiniosis [33]. Similar results have been investigated in other studies [31,32].

*Enterococci faecium* are part of the normal microflora of the gastrointestinal tract of humans and many vertebrates and play a crucial role in providing mucosal colonization resistance. Wang et al. (2008) isolated an *E. faecium* ZJ4 from the intestinal tract of a healthy piglet and investigated its significant effect on the body weight of tilapias [37].

*Micrococcus*. *Micrococcus* is a genus of gram-positive bacteria. The role of *Micrococcus luteus* in human and animal disease is minimal, subsequently, it can be used as a probiotic. In a study with tilapia, *M. luteus* enhanced fish growth and health in vivo [38].

*Further perspectives*. Modern fish farming is based on intensive technologies, including closed water supply plants, which feature high planting density in limited areas, which greatly increases the risk of infection of fish with pathogens of dangerous infections. Feeding probiotic preparations are widely used in animal breeding practice, which allows for improving the existing systems of breeding and feeding of farm animals, and becomes an important component of the modern rational feeding of animals.

The review of available literature revealed the promising effects of probiotics on the growth performance of various fish. This helps to reduce feed costs and improve the economic efficiency of horseshoe fish farming. The analysis of potential probiotics has shown that their further study and implantation into aquaculture can contribute to the increase of fish yield. However, research on the potential effect of probiotics lacks in fish; therefore, it still needs further exploration in various fish species.

**Table 1**

**Different species of microorganisms and their beneficial effects on growth performance and immunity of fish**

№	Probiotic candidates Gram-Positive Bacteria	Aquatic species	Effects	References
1	<i>Lactobacillus helveticus</i>	<i>Carassius auratus</i>	antimicrobial activity, improved health, reduced mortalities	[11]
2	<i>Lactobacillus plantarum</i> 426951	<i>Oncorhynchus mykiss</i>	improved growth performance and immune status	[33]
3	<i>Lactobacillus plantarum</i> CCFM639	<i>Tilapia</i>	Enhances growth performance, feed utilization, and antioxidant ability	[31]
4	<i>Lactobacillus plantarum</i> (PTCC no. 1058)	Siberian sturgeon <i>Acipenser baerii</i>	improve growth performance and feed utilization, immunological parameters	[32]

5	<i>Bacillus coagulans</i> (MTCC 9872)	<i>Cyprinus carpio</i> fry	improve growth, feed utilization, non-specific immune responses and disease resistance	[24]
	<i>Bacillus licheniformis</i> (MTCC 6824)			
	<i>Paenibacillus polymyxa</i> (MTCC 122)			
6	<i>Enterococcus faecium</i> ZJ4	Tilapia <i>Oreochromis niloticus</i>	Improved growth performances and the immune responses	[37]
7	<b>Micrococcus luteus</b>	<b>Nile tilapia</b>	enhanced the fish growth and health	[38]
8	<i>Bacillus</i> sp.	<i>Cyprinus carpio</i>	increased the growth performances and digestive enzyme activities	[20]
9	<i>B. coagulans</i> B16	Tilapia <i>Oreochromis niloticus</i>	enhance immune and health status, growth performance	[39]
10	<i>Bacillus subtilis</i>	<i>O. niloticus</i>	significant improvement in growth parameters	[12]
11	<i>B. pumilus</i>	Tilapia <i>Oreochromis niloticus</i>	enhanced growth	[40]
12	<i>Lactobacillus rhamnosus</i> IMC 501	clownfish larvae	the higher body weight, earlier metamorphosis, and lower deformity incidence	[29]
13	<i>Lactobacillus acidophilus</i>	<i>Cyprinus carpio</i>	Improved growth performance and total heterotrophic microbial load	[41]
14	<i>Lactobacillus</i> spp.	<b>gilthead sea bream</b> <i>Sparus aurata</i> , L.	increase growth parameters and digestive enzyme activities	[42]
15	<i>B. subtilis</i>	<b>Nile tilapia</b> ( <i>Oreochromis niloticus</i> )	<b>Improved growth, survival and body composition</b>	[23]
16	<i>L. rhamnosus</i>	red sea bream	improvements in growth performance, feed utilization, immune response and oxidative status	[30]
17	<i>L. lactis</i> ssp. <i>lactic</i> ST G45	Siberian sturgeon ( <i>Acipenser baerii</i> )	promote growth performance and boost some immune response	[43]

18	Lactobacillus plantarum	olive flounder <i>Paralichthys olivaceus</i>	enhanced the growth, blood biochemical constituents, and nonspecific immunity	[44]
	Lactobacillus brevis			
	Lactobacillus acidophilus			
	Bacillus subtilis			
19	<i>Lactobacillus delbruekii</i> subsp. <i>Bulgaricus</i>	<b>rainbow trout</b> <i>Oncorhynchus mykiss</i>	growth performance by improving digestive enzyme activity, gut microflora and growth gene expression	[36]
	<i>L. acidophilus</i>			
20	<i>Lactobacillus acidophilus</i>	<b>Snakehead</b> ( <i>Channa striata</i> )	Improved growth and the expression of immune regulatory genes	[28]
21	<i>L. lactis</i>	<b>Rohu</b> <i>L. rohita</i>	Better growth, protein efficiency ratio, nutrient retention and digestibility	[45]
	<i>B. subtilis</i>			
22	<i>L. lactis</i> L19	<b>snakehead fish</b> ( <i>Channa argus</i> )	promoted growth performance, humoral immunity, regulated immune-related genes expression and disease resistance	[34]
	<i>E. faecalis</i> W24			
23	<i>Bacillus subtilis</i>	<b>starry flounder,</b> <i>Platichthys stellatus</i>	growth performance, non-specific immune responses and disease resistance	[15]
	<i>Bacillus licheniformis</i>			
24	<i>Lc. lactis</i> WFLU12	<b>olive flounder,</b> <i>Paralichthys olivaceus</i>		[35]

## Conclusion

Antimicrobial activities of probiotic applications reduce the mortality rate by improving their health by supporting the immune responses and oxidative status of fish. However, it increases the growth of fish by improving the feed conversion ratio. In conclusion, it is thought that probiotics and their derivatives in aquaculture can be used reliably instead of antibiotics in healthy fish rearing and organic fishers, and this situation can be supported by future studies.

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### **Пробиотиктер және оларды балықтардың өсуі мен иммунитетін жақсарту үшін аквакультурада қолдану**

**Аңдатпа.** Азықтың қорытылуы мен сіңімділігін арттыру, жануарлардың өсуі мен дамуын ынталандыру, спецификалық емес иммунитетті арттыру үшін ферменттік, пробиотикалық, пребиотикалық және аралас ферменттік-пробиотикалық препараттар, сондай-ақ фитокомпоненттермен байытылған кешенді пробиотикалық препараттар қолданылады.

Жасанды өсірілген балыққа арналған пробиотиктер-бұл денсаулықты, өнімділікті және өсуді сақтау үшін дәстүрлі түрде аквакультура ауылшаруашылық жүйелеріне қосылатын пайдалы бактериялар және тірі микробиалды қоспалар. Сондықтан аквакультурадағы перспективалы бағыт пробиотикалық дақылдармен жемді пайдалану болып табылады. Пробиотиктерді қолдану денсаулықтың әртүрлі мәселелерін шешумен, нақтырақ айтатын болсақ ас қорыту тиімділігін арттырумен, өсу мен дамуды ынталандырумен байланысты. Пробиотиктер иесінің ағзасына оң әсер ете отырып, ас қорытуды, биологиялық мәртебені, иммундық реакцияны қалпына келтіруге ықпал етеді және вакцинацияның тиімділігін арттырады. Пробиотиктерді қолдану жануарлардағы ауруларды емдеуге кететін шығындарды едәуір азайтады, өнімділікті арттырады және өнім сапасын жақсартады. Соңғы жылдары балық шаруашылығында әртүрлі пробиотиктерді қолдану бойынша бірқатар зерттеулер жүргізілді. Бұл шолу қолданыстағы әдебиеттерді жан-жақты зерттей отырып, пробиотиктер ретінде грам-позитивті бактерияларды ықтимал қолдану тақырыбын жалпылауға бағытталған. Пробиотиктердің биологиялық тиімділігі туралы түсініктердің кеңеюімен және жасушалардың құрылымдық элементтері мен олардың метаболиттері кейбір жағдайларда кем дегенде тиімді екенін дәлелдеу болып табылады. Нәтижесінде әр түрлі балықтардың өсуі мен иммунитетін жақсарту үшін бактериялардың әртүрлі түрлері, соның ішінде бациллалар, сүт қышқылы бактериялары, микрококстар қолданылды. Алайда, бұл зерттеу аквакультурадағы пробиотиктердің жалпы балық өнімділігіне оң әсер ететінін көрсететін ғылыми әдебиеттерге шолу жасалды.

**Түйін сөздер:** пробиотиктер, аквакультура, балық, бактериялардың штамы, шолу, сүт қышқылды бактериялар, азық.

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## **Пробиотики и их применение в аквакультуре для улучшения роста и иммунитета рыб**

**Аннотация.** Для повышения усвояемости кормов, стимулирования роста и развития животных, повышения неспецифического иммунитета используются ферментные, пробиотические, пребиотические и комбинированные ферментно-пробиотические препараты, а также комплексные пробиотические препараты, обогащенные фитоконпонентами.

Пробиотики для искусственно выращенной рыбы - это полезные бактерии и живые микробиологические добавки, которые традиционно добавляются в сельскохозяйственные системы аквакультуры для поддержания здоровья, продуктивности и роста. Поэтому перспективным направлением в аквакультуре является использование кормов с пробиотическими культурами. Использование пробиотиков связано с решением различных проблем со здоровьем, в частности, с повышением эффективности пищеварения, стимуляцией роста и развития. Пробиотики, положительно воздействуя на организм хозяина, способствуют восстановлению пищеварения, биологического статуса, иммунного ответа и повышают эффективность вакцинации. Использование пробиотиков значительно снижает затраты на лечение заболеваний у животных, повышает продуктивность и улучшает качество продукции. В последние годы был проведен ряд исследований по использованию различных пробиотиков в рыболовстве. Этот обзор направлен на обобщение темы возможного использования грамположительных бактерий в качестве пробиотиков при всестороннем изучении существующей литературы. С расширением представлений о биологической эффективности пробиотиков и доказательством того, что структурные элементы клеток и их метаболиты в некоторых случаях по крайней мере эффективны. В результате различные виды бактерий, в том числе бациллы, молочнокислые бактерии, микрочки, использовались для улучшения роста и иммунитета различных рыб. Однако в этом исследовании был проведен обзор научной литературы, показывающий, что пробиотики в аквакультуре положительно влияют на общую продуктивность рыб.

**Ключевые слова:** пробиотики, аквакультура, рыба, штамм бактерий, обзор, молочнокислые бактерии, корм.

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