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PROBIOTICS BASED ON LACTIC ACID BACTERIA FOR AQUACULTURE

Aquaculture (especially fish farming) provides billions of people with nutrients: amino acids, vitamins, minerals, and proteins. Aquaculture of commercial species (rainbow trout, common carp, grass carp, pacific white-legged shrimp, etc.) are growing rapidly and accounts for about a third of the global fish production. It is a major alternative to reducing wild populations that are unable to meet growing food demand. Lactic acid bacteria (LAB) of the genera Lactococcus, Lactobacillus, Lactiplantibacillus, Enterococcus, Leuconostoc, Pediococcus, Streptococcus, Carnobacterium, and Weissella belong to the normobiota of the gastrointestinal (GI) tract of most fish. They play an important role as they stimulate the synthesis of digestive enzymes, prevent intestinal disorders, improve the immune response of the macroorganism, increase the barrier capacity of the mucous membrane due to the colonization of the fish GI tract, activate the resistance to the development of bacterial and viral infections due to the production of a wide range of antimicrobial substances (bacteriocins, organic acids, hydrogen peroxide, etc.). Therefore, Lactobacilli are classified as potential probiotic strains for aquaculture. The combined use of lactic acid bacteria with feed increases their nutritional value, as microorganisms produce a wide range of digestive enzymes that actively participate in the process of digestion and decomposition of feed, as well as positively affect the growth of individuals and stimulate their reproductive activity, which undoubtedly contributes to the active introduction of commercial probiotics based on LAB strains. As a result of growing aquaculture, there are many problems such as stocking density, infectious diseases, excessive use of antibiotics, water pollution, which can be solved with the help of probiotic lactic acid bacteria. Therefore, the review presents current literature data on the use of lactic acid bacteria strains as probiotic preparations for aquaculture, their species composition, probiotic effect on the host, the mechanism of action of probiotics on aquaculture, and the methods for delivery to the macroorganism.

Keywords: *lactic acid bacteria, aquaculture, probiotic strains, fish, probiotics.*

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Aquaculture is the third most important source of animal protein [1, 2] in Ukraine after livestock and poultry. The development of this domestic industry is an extremely promising sector of agriculture. First of all, it is so due to the growing demand for fish in Ukraine and the need for obtaining safe products at affordable prices. However, currently Ukraine imports the vast majority of fish products consumed. Growing demand for fish is a global trend [3]. Thus, due to natural resources [4], human and educational potential, Ukraine has all the opportunities for the development of aquaculture and is able not only to meet domestic consumption needs but also to enter foreign markets. Therefore, development and implementation of all kinds of innovations in this field will contribute to its development.

Aquaculture is an artificial cultivation of fish and other aquatic products — hydrobionts. It is carried out in fully or partially controlled conditions: in cages, internal water reservoirs, water recirculation systems, etc. As a result of growing aquaculture, there are many problems that need solving. One of such problems is that aquaculture stocking density affects the growth rate, survival rate, water quality, and feed consumption rate. When stocking density increases, the level of stress increases as well, which subsequently leads to decrease in the growth rates. However, at a low population density, fish may not form shoals, and individuals become less protected. In addition, high stocking densities and overfeeding have been shown to contribute to water pollution, which increases sensitivity to infectious diseases and diseases of parasitic origin. Correctly selected density of individuals is a critical factor for a certain species and contributes to optimal farming [5].

Another problem of aquaculture is infectious diseases. In connection with the increase in the volume of aquaculture production, the number of individuals infected with bacterial, fungal, and viral pathogens is increasing as well, which leads to economic losses and as a result — to deterioration of environmental conditions [6]. Therefore, the screening of probiotic strains of microorganisms *in vitro* is focused on search for active strains that facilitate inhibition of the growth or lead to the death of pathogens in the aquatic environment [7].

Infectious diseases of bacterial origin are the main cause of disease and mortality of fish in isolated water reservoirs [8]. Bacterial diseases such as streptococcosis, vibriosis, intestinal septicemia, columnaris, aeromonosis, edwardsiellosis, and mycobacteriosis have increased significantly in aquaculture systems over the past decades. Among bacterial pathogens, the greatest threat to aquaculture is provided by genera *Vibrio*, *Flavobacterium, Edwardsiella, Streptococcus, Mycobacterium Aeromonas,* and *Renibacterium* [8], which pose a threat to fisheries and require effective control strategies in this industry [9].

Streptococcosis caused by *Streptococcus iniae* can affect various types of freshwater and marine fish [10]. The pathological process develops as a result of synthesis of exo- and endotoxins. In the industrial conditions, young rainbow trout and Atlantic salmon are quite susceptible to this pathogen. Fish farms use erythromycin for treatment of infected individuals. However, the effective use of probiotic microorganisms has been demonstrated as well, in particular *via* the addition of strain *E. faecium* ABRIINW.N7, which reduces the development of streptococcosis in red hybrid tilapia [11] and promotes an increase in fish weight [12].

Vibriosis is one of the most common bacterial diseases (*Vibro*) in marine and brackish water aquacultures. Development of the infectious process is facilitated by high pH ≥ 8, water temperature ≥15 °C, low O_2 content and water pollution with organic substances. It is known that high density of individuals, decrease in immunity, and stressful conditions contribute to the active development of vibriosis [13]. The disease usually has an acute course, infected fish almost always die without showing clinical signs. The chronic form is quite rare and occurs after treatment with antibiotics. According to the literature data, the addition of *L. plantarum* strain to the feed of Pacific white shrimp significantly reduces the risk of infection with *Vibrio spp.* [14], and use of strain *L. pentosus* suppresses the development of *V. vulnificus, V. rotiferianus, V. campbellii,* and *V. parahaemolyticus* [15].

Mycobacteriosis is an infectious disease of many freshwater, marine, and aquarium kinds of fish [16]. The most contagious species are *Micobacterium marinum* and *M. fortuitum*. Kanamycin is used to treat sick individuals. However, the active use of antibiotics leads to increase in the antibiotic resistance in pathogens, decrease in fish immunity due to the effects on gut microbiota, and risks associated with contaminated food [17]. Therefore, probiotics and postbiotics are actively used, which is a more environment-friendly alternative for control and prevention of infectious diseases in aquaculture. Lactic acid bacteria and bacteria of the genus *Bacillus* are often used thanks to their safety and production of hydrolytic enzymes that increase the nutrients utilization level; in particular, species of *P. acidilactici* [18] helps to reduce the level of mycobacteria in sick fish [19].

Edwardsiellosis, which is caused by bacteria of the *Enterobacteriaceae* family, leads to significant economic losses in the cultivation of catfish, eels, brook trout, and largemouth bass. Young individuals are especially susceptible. Low-quality and substandard feeding, an increase in water temperature (≥30°C), and a high content of organic substances in the aquatic environment contribute to the development of the disease. Mortality of sick individuals reaches 90%. Chang have shown that the use of *E. faecium* SF68 and *B. toyoi* strains promote inhibition of abovementioned pathogens' development and contributed to reduction of mortality of the European eel [20].

Bacterial kidney disease is one of the most common infectious diseases of salmonids grown in both fresh and sea water. The causative agent is *Renibacterium salmoninarum,* which affects only salmonid fish, mainly individuals older than six months. Rainbow trout is the least susceptible to this pathogen unlike the brook trout. Antibiotics used to treat bacterial kidney disease are sulfamethazine, erythromycin, and sulfamerazine [21].

Infectious diseases of viral origin. In aquaculture, both farmed individuals and wild aquatic animals in the same water column are exposed to the same viral challenges. However, the diseases of viral etiology transmitted by wild aquatic animals, whose density is often not sufficient to support the circulation of the virus, are extremely dangerous for the growth of farmed aquaculture due to the high density of individuals, which in turn contributes to chronic stresses [22]. Among the viruses that can affect aquaculture, the most common are viral hemorrhagic septicemia, infectious necrosis of hematopoietic tissue, infectious necrosis of the pancreas, infectious salmon anemia virus, lymphocystosis virus, and aquabirnavirus [23]. Today, farmers partially control infectious diseases through the heavy use of antibiotics such as amoxicillin, oxytetracycline, gentamicin, tetracycline, and cefazolin, and/or chemicals such as formalin, hydrogen peroxide, and sodium chloride.

Infectious pancreatic necrosis and infectious hematopoietic necrosis of tissue are highly contagious diseases of salmonid fish, registered in both freshwater and marine aquaculture. An acute outbreak of them is accompanied by a sudden mass death of fish, often without external manifestations. In the chronic course of the disease, the symptoms are less pronounced, and the death of fish is stretched over time.

To combat viral diseases of fish, considerable attention of scientists is focused on the search for probiotic bacteria with antiviral activity [24]. Back in 1988, Kamei and co-authors showed that bacteria isolated from freshwater hatchery farms for salmonids have antiviral activity against the infectious hematopoietic necrosis virus. Compositions of LAB strains help to increase resistance of fish to diseases of viral etiology. The use of a dietary feed supplement with the commercial probiotic «Lactobacil», alone or mixed with Sporolac, in the diet of flounder infected with lymphocystis virus increased resistance to the disease.

Infections of fungal etiology contributing to development of saprolegniosis, one of the most common diseases in freshwater fish, lead to significant financial losses [25]. Today, antifungal antibiotic drugs are used for treatment. Aflatoxins of *Aspergillus flavus* and *A. parasiticus* strains are one of the main feed contaminants [26]. It has been shown that aquaculture is sensitive to the effects of aflatoxins, especially aflatoxin B1 which suppresses the immune system of a macroorganism and makes it more susceptible to infectious diseases [27], and also exhibits carcinogenic and mutagenic effects [28]. Contamination of fish feed with aflatoxin has a negative effect on fish production, due to accumulation of toxins in adult individuals during contaminated feed consumption [29]. One of the possible ways to solve this problem is the use of LAB probiotic strains. In the work of Martinez M. P. and co-authors (2017) [12], it was shown that *Pediococcus sp.* strain is capable to adsorb and break down aflatoxin B1 at a concentration of 20 ng/mL.

Another problem of aquaculture is the widespread use of antibiotics. Lactic acid bacteria's strains can be an ecological alternative to antibiotics. For a long time, antibiotics and chemotherapeutic drugs were added to the basic diet to improve the reproductive capacity, as well as to increase the growth of aquaculture [30]. However, in the Regulation of the European Union from 2003 No. 1831/2003, it was stated: «Antibiotics other than coccidiostats or histomonostats should not be authorized as feed supplements.» This regulation forced aquaculture producers and scientific community to look for an alternative antibiotics, which were probiotics. Another not less important problem in the use of antibiotics as a therapeutic and preventive means against infectious diseases in fish farming was the appearance of antibiotic-resistant microorganisms and the change in the aquaculture microbiota [31]. In addition, the widespread use of antibiotics in aquaculture affects the sale of fish to the final consumer, because a certain concentration of chemicals is stored in the fillet for a long time [27], which also stimulates the search for probiotic strains for the prevention and control of infectious diseases [32].

Replacement of antibiotics with effective and environmentally safe probiotics has gained recognition because it allows one to avoid development of antibiotic-resistant microorganisms in aquaculture and to reduce their number in fish meat used in the food industry [33].

Probiotic cultures include microorganisms that are not pathogenic and cause a positive effect on the host organism by improving immunity, increasing antagonistic activity and stimulating the fish growth, improving digestion and increasing the value of feed [34]. Today a wide range of probiotics are offered for aquaculture including gram-negative and gram-positive bacteria, bacteriophages, and various yeast species isolated from different ecological niches [35]. In recent years, strains of lactic acid bacteria have been actively used as probiotics in aquaculture as they have the GRAS status (safe for consumption). They possess a wide range of antagonistic activity against conditionally pathogenic, pathogenic microorganisms antifungal and antiviral effects [36] due to the direct activity of cells or the synthesis of metabolites (organic acids, bacteriocins, hydrogen peroxide, short-chain fatty acids, diacetyl, lysozymes, and siderophores) [37].

According to FAO/WHO, microorganisms in commercial probiotic preparations must have taxonomic identification through safety studies (for non-toxicity, non-pathogenicity, etc.) and results of *in vitro* and *in vivo* functional studies [38]. The European Food Safety Agency (EFSA) proposed the Qualified Presumption of Safety (QPS) status, i.e., a system for assessing the safety of certain groups of microorganisms used in food/feed and in the production of food/feed supplements [33]. A significant part of LAC strains has the QPS status according to the EFSA regulation [39].

Mechanism of the action of probiotic strains of lactic acid bacteria on aquaculture. Mechanisms of probiotics action in aquaculture are divided into: secretion of metabolites with antagonistic activity; adhesion and colonization of the intestinal mucosa, improvement of nutrients bioavailability, and detoxification of toxic compounds; increased antagonism to pathogens in both humans and animals; synthesis of enzymes to improve digestion; and antiviral effect and improvement of water quality due to modulation of aquatic microbiota [37, 40].

Secretion of metabolites with antagonistic activity. Strains of LAB have antagonistic activity against causative agents of infectious diseases due to synthesis of a wide range of exometabolites — organic acids, diacetyl hydrogen peroxide, and bacteriocins [41]. Exometabolites of microorganisms are able to activate the immune system of animals, which increases their resistance to bacterial, viral, and fungal diseases. The production of mucus containing antimicrobial substances (lysozyme, peroxidase) is the first defense reaction to stress or disease development [42]. The use of *L. plantarum* strains has been shown to increase the antibacterial activity, lysozyme and peroxidase activity of skin mucus in Nile tilapia [43].

Adhesion and colonization of the intestinal mucosa. In aquaculture, the probiotic activity of microorganisms occurs due to the ability to colonize the gastrointestinal tract and immunostimulation of the host organism. One of the first probiotic effects is adhesion of probiotic microorganisms to intestinal cells, resulting in competition with pathogens for the gastrointestinal tract of aquaculture. Probiotic strains of lactic acid bacteria can act as immunostimulants, stimulating the host's immune system [37]. They can not only improve the absorption of nutrients and increase the body's resistance to stress, but also increase the fertility of host organism.

Increase of immune status. The immunological effect of the probiotic strain on the host is strain-specific. Addition of probiotic microorganisms to aquaculture helps to increase the number of leukocytes, lymphocytes, monocytes, and erythrocytes [44] and improves the bactericidal properties of plasma [45]. In addition, they increase total globulin [46], albumin, and peroxidase. It was shown that the autochthonous probiotic strain *L. plantarum* increases the mRNA level of the main anti-inflammatory cytokine IL-10 in uninfected primary intestinal cells. Aubin et al. used *P. acidilactici* strains as an alternative treatment for vertebral column compression syndrome in rainbow trout. Studies of the effect of probiotic *L. plantarum* on tilapia have shown stimulation of its growth, increase in the level of the humoral link of immune protection, and improvement of mucous barrier and resistance to diseases in fish [47].

Effect on the antioxidant system. Like most organisms, fish have a non-enzymatic antioxidant defense system (synthesis of vitamins E/C, glutathione, and thioredoxin) and an enzymatic antioxidant defense system (synthesis of superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase) [48]. For the normal functioning of a macroorganism, a balance between the formation of reactive oxygen species and their neutralization is necessary, the lack of which can lead to oxidation of biological macromolecules and cells' damage [49]. In the fish organism, a shift in this balance toward an increase in ROS amount can be caused by changes in temperature (hyper- or hypothermia) [50], fluctuations in the concentration of O_2 in the habitat [51], insufficient nutrition or the presence of pesticides and heavy metals in the water [52]. The addition of probiotic microorganisms to food supplements has been found to improve the antioxidant system in various fish species [53]. In particular, *L. plantarum* strains possess antioxidant activity [54] and significantly reduce oxidative cell damage when added to freshwater fish feed.

Improvement of the nutrients bioavailability and synthesis of enzymes to improve digestion process. Probiotic strains take an active part in digestion processes through production of enzymes — proteases, lipases, and carbohydrolases [37]. In addition, they improve feed digestion by aquaculture due to the enhancement of digestive enzymes — amylases, proteases, and alginate lyases [55], which supports high growth rates and survival of individuals. Addition of *L. lactis* (10^8 CFU/g) to shrimp feed has shown to increase protease and amylase activities [56].

Improvement of water quality due to modulation of aquatic microbiota. Studies have proven the effect of probiotic microorganisms on water quality due to stabilization of pH of the environment, the reduction of toxic and organic substances and pathogenic microorganisms and viruses. In addition, probiotics play an active role in the formation of water microbiota [57]. It was also established that microorganisms of the water environment contribute to the increase in the amount of dissolved oxygen and decrease in nitrates [58]. Addition of probiotics has been shown to reduce metabolic waste levels during transportation of *Paracheirodon aexlrodi* [59].

When choosing and using probiotics based on LAB strains for aquaculture, the following aspects should be considered: the source of strains, species composition of microorganisms, method (through water/feed) and frequency of administration (single, multiple, or combined).

Source of probiotic microorganisms isolation and their speciescomposition. A significant part of probiotic strains used for aquaculture is autochthonous cultures isolated from terrestrial ecosystems. They have a positive effect on macroorganisms (stimulation of growth, suppression of foreign microbiota, etc.) [60]. However, certain disadvantages may arise for aquaculture, in particular, lack of data on strain's effect on the fish gastrointestinal tract, possible interaction of probiotic culture with microbiota of the target organism [61], and ability to survive under a wide temperature range.

Microbiota of the fish GI tract is divided into allochthonous (resident) and autochthonous (transient in the fecal stream) microbes. It plays a significant role in protecting aquaculture from infectious processes stimulating the host's immune response and digestive processes [62]. Allochthonous microorganisms play a key role in nutrition, metabolism, and physiology of the macroorganism due to the faster colonization of aquaculture's intestine and restoration of its homeostasis [63].

A significant part of scientists is focused on the search for lactic acid bacteria strains isolated from the fish intestines [64]. This can enable to develop species-specific probiotic preparations adapted to the intestinal environment of target species [65] with pronounced antimicrobial activity against the most common pathogen, namely bacteria of the genera *Vibrio, Aeromonas,* and *Pseudomonas*.

Lactic acid bacteria are able to colonize the fish intestine and have a therapeutic effect on the host's macroorganism [37]. In the works of other authors, it was demonstrated that fish normobiota includes genera *Leuconostoc, Lactococcus, Lactobacillus, Streptococcus, Enterococcus, Weissella Pediococcus,* and *Carnobacterium* [37].

Bacteria of the *Lactobacillus* genus include homo- and heterofermentative acid-resistant and facultatively anaerobic microorganisms. Scientific studies have shown that the GI tract of various fish species contains *Lactobacilli*, in particular *L. aviarius, L. arafinosus, L. brevis, L. crispatus, L. farciminis, L. gallinarum, L. johnsonii, L. reuteri,* and *L. sakei* [66]. Bacteria of the *Lactococcus spp*. genus were first isolated from common carp by Cai et al. [67]. Studies conducted in recent years have demonstrated that this genus is represented by many species in the gastrointestinal tract of aquaculture, namely *L. lactis garvieae, L. lactis subs P. cremoris, L. piscium,* and *L. raffinolactis* [68]. Strains of the *Leuconostoc* genus are heterofermentative microorganisms resistant to vancomycin. This species belongs to the GI tract normobiota of rainbow trout, Atlantic salmon, and arctic char [68]. Homofermentative microorganisms of this genus were first isolated from the aquaculture's intestines in the late 90s [69]. Representatives of the genus *Enterococcus* belong to the GI normobiota of aquaculture. Strains of *E. faecalis* and *E. faecium* were isolated from rainbow trout, anglerfish, and European sea bass [70]. The *Peodiococcuss* genus includes homofermentative gram-positive cocci arranged in pairs or tetrads. They have ability to grow in a wide range of pH, temperature, and osmotic pressure [71], due to which they actively colonize the GI tract of aquaculture. The strains produce organic acids and bacteriocins that inhibit a number of gram-positive and gram-active microorganisms. Martinez et al. have shown that species *P. pentasoceus* and *P. acidilactici* constitute a significant part of the microbiota of rainbow trout [72]. *Carnobacterium* consists of 10 species isolated from finfish intestine. **For the first time, carnobacteria were isolated from the gastrointestinal tract of wild Atlantic salmon.** Carnobacteria are the most prevalent of the autochthonous LAB in salmonids. The genus *Weissella* includes 19 species of obligately heterofermentative microorganisms which produce lactic and acetic acids as the main metabolic products. To date, information on the effectiveness of *Weissella* bacteria as probiotics in aquaculture is quite limited. In 2014, *W. confusa* was first isolated from the gastrointestinal tract of sea bass by Rengpipat et al. [73], and in 2017 autochthonous and allochthonous microorganisms of this genus were isolated from rainbow trout by lyons et al. [74]. As for the strains of genus *Bifidobacterium*, which are widely represented in the normobiota of endothermic animals, they are not isolated so often from the gastrointestinal tract of fish. However, Boonanuntanasarn et al. have found a large number of *Bifidobacteria* in Nile tilapia fry [75]. The quantitative and qualitative compositions of fish normobiota depend on the season, location of fishing area, and sampling zone.

To date, probiotic preparations based on lactic acid bacteria of genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Enterococcus* have become widespread [76]. *P. acidilactici* species was first authorized in the European Union for aquaculture only about 10 years ago [33]. *Pediococcus spp.* strains isolated from the GI tract of rainbow trout have a high antagonistic activity to the pathogenic microorganisms *E. coli, P. aeruginosa, S. typhimurium,* and *S. aureus* [71].

The list of probiotic strains of lactic acid bacteria that are actively used in aquaculture is given in Table 1.

Methods for administration of probiotic strains (water/feed) into aquaculture. There remains an important issue of development of new strategies to stabilize the species composition of the host's microbiota, in particular the use of food supplements with probiotic microorganisms. In aquaculture, two most common ways of using probiotics are adding probiotic microorganisms to the fish feed and adding probiotic microorganisms to the water environment.

Addition of probiotic strains of lactic acid bacteria to aquaculture feed. The use of carefully designed and selected feeding strategies is one of the main factors of successful commercial aquaculture reproduction. The diet components are balanced according to the nutritional needs of a certain fish species. In addition, farmers add biologically active additives that ensure increased productivity and normalization of homeostasis [86]. Nowadays the generally accepted practice in the industrial aquaculture is the use of balanced feed in a complex with probiotics, which ensures a positive effect on aquaculture, consumers, and farmers as the development of infectious diseases reduces, fish immunity increases, and growth and meat quality improve [34]. A variety of ingredients are used in the production of feed, including fish meal, fish oil, soybean oil, cake, rice bran, wheat flour, corn, meat and bone, calcium phosphate, salt, and vitamin premixes [87].

One of the possible options for the use of probiotic cultures in aquaculture is the use of granular feed or live feed (rotifer, artemia). Thus, according to the literature data, a positive effect in

Table 1. **Overview of lactic acid bacteria and their beneficial effects on aquatic organisms**

Probiotic strains of lactic acid bacteria	Beneficial effects	Mode of application	Targeted species	References
L. plantarum	Reduced pathogen load Enhanced immunity Significantly up-regulated expression of cytokine genes, IL-4, IL-12 and IFN-γ	Dietary supplement	Silver catfish, Rhamdia quelen, Japanese flounder, Nile tilapia Oreochromis niloticus	[64]
Lactobacillus sp.	Reduced pathogen load, provided protection against Aeromonas hydrophilla	Dietary supplement	Zebrafish	[77]
L. acidophilus	Improved water quality	Water supplement	African catfish	[78]
L. rhamnosus	Reduced pathogen load in culture tank	Water supplement	Portunus pelagicus	[79]
L. rhamnosus	Enhanced immunity and reduced disease susceptibility Improved blood quality	Dietary supplement	Oncorhynchus mykiss	[80]
L. sakei	A non-significant decrease in the cumulative mortality	Dietary supplement	Rock bream	[81]
L. pentosus	Improved immune response and survival rate	Dietary supplement	Japanese eel	[82]
L. brevis / L. acidophilus	Significantly lowered mortality	Dietary supplement	Tilapia	[83]
E. faecium	Improved water quality and enhanced immunity	Water supplement		[11, 12]
P. acidilactici	Enhance immunity and reduced disease susceptibility. Reduced pathogen load	Water supplement	Psetta maxima	[84]
P. pentosaceus	Significantly decreased cumulative mortality	Dietary supplement	Orange-spotted grouper	[72]
Rhodopseudomonas palustris, L. plantarum, L. casei, S. cerevisiae	Reduced nitrate load, maintained water pH and enhanced dissolve oxygen concentration	Water supplement	Litopenaeus vannamei	[58]
L. fructivorans *L. plantarum*	Enhanced colonization on the gut epithelial surface and significantly reduced mortality rate during larval rearing and fry culture	Dietary supplement	Sparus aurota	[85]

the enrichment oflive feed for aquaculture was shown, in particular with artemia, [88], rotifers [45], and copepods [89]. A significant number of probiotic bacteria enters the fishlarvae through live feed [90], and, as a result, the growth of larvae improves, the survival rate of individuals increases [91], the level of pathogenic bacteria decreases, and the immune response of fish improves [92]. The use of probiotic microorganisms in the form of capsules for live feed ensures their survival and reproduction and effectively enters the host organisms.

Encapsulation of probiotic microorganisms is an effective and modern way of introducing the preparations into aquaculture. At the same time, cells in a high density are encapsulated into the colloidal matrix, e.g. alginate, chitosan, carboxymethyl cellulose, or pectin, which contributes to the chemical and physical protection of microorganisms. Encapsulation in alginate granules has been shown to protect bacteria from exposure to low pH and GI enzymes [93] and to help to performa probiotic function in the GI tract of fish.

In the studies of Zhang et al., addition of live cells to the feed (1×10^7 CFU/g, strain *L. delbrueckii*) was shown to increase the expression of IL-10 and TGF-β genes [94]. In other studies, fish feed was supplemented with *L. plantarum* VSG3 in the amount of 0, 10^6, 10^8, 10^{10} CFU/g for 60 days, after which it was infected with *A. salmonicida*. The results of the study have shown stimulation of the organism immune response and a decrease in the intestinal inflammation level after infection with the specified pathogen. In the study of influence of a dietary supplement containing *W. cibaria* strain (1.18×10^7 CFU/g), an increase in the productivity and hematoimmunological parameters of surubins was demonstrated [95].

It was shown that the addition of the *L. acidophilus* strain to the diet of salmon in a lyophilized state has a growth-stimulating effect on the microorganism [96]. When using the autochthonous strain of *L. lactis* as a feed supplement for young *Rhamdia quelen (silver catfish)*, a higher immunostimulating effect was established in comparison with the allochthonous strain of *L. plantarum*. Addition of probiotic strain of *L. acidophilus* (3.01×10^7 CFU/g) to the diet of African catfish and *L. rhamnosus* JCM 1136 (10^9—10^{11} CFU/g) for rainbow trout [97] led to an immunoglobulin level increase. The use of the probiotic strain *P. pentosaceus* in a complex with food products led to sharp reduction in the mortality of perch infected with *V. anguillarum*. It was also shown that addition of probiotic microorganisms (*L. mesenteroides/L. lactis/L. sakei*) to fish feed reduces the severity of furunculosis caused by the pathogenic strain *A. salmonicida* thanks to the enhancement of humoral and cellular immune response.

Addition of LAB probiotic strains to the aquatic environment of aquaculture. The addition of probiotic microorganisms to the aquatic environment is a universal way to deliver a target product suitable for any age of aquaculture (from fingerling to adult individuals). Probiotic microorganisms are widely used in freshwater aquaculture [98], brackish water, and sea water [99]. It was shown that LAB strains of different econiches are able to maintain their vital activity in seawater for 2—4 days after inoculation [100]. The addition of probiotics to the aquatic environment is extremely relevant when rearing aquaculture larvae in cold water, as the GI tract, gills, and skin are the primary entry routes for bacterial pathogens [101].

Probiotic microorganisms have become widely used in aquaculture, not only due to the improvement of the immune status of fish, crustaceans, shrimps and the reduction of infectious diseases development, but also as a result of water quality improvement due to increasing the amount of dissolved oxygen, reducing nitrates, and stabilizing pH of water [58].

At the larval stage of development, the use of feed has certain limitations due to the insufficient development of the fish digestive tract. Addition of probiotics to the water to stimulate growth and reduce the risk of infection in aquaculture is possible from the moment of the larvae hatch. It is noted that the addition of microorganisms to water contributes to better penetration of bacteria into the fish body especially in salt water [85, 8]. Thus, it has been shown that introduction of the probiotic *P. acidilactaci* strain into the aquatic environment promotes the rapid colonization of GI tract of turbot larvae (*Psetta maxima*), and exometabolites of this strain have antimicrobial activity against the pathogenic strain *V. splendidus*. The addition of the probiotic *L. plantarum* strains to the cod growth medium contributed to colonization of the GI tract of the larvae, and it was established that mentioned strains consisted 70% of the intestinal microflora. The introduction of *L. plantarum* culture into an incubator with eggs and larvae of Atlantic halibut increased larval survival. The addition of a probiotic preparation based on *L. plantarum* and *L. casei* strains to the aquatic environment of shrimp led to reduction in the levels of nitrates and organic residues in water [59]. Also, it was shown that the growth period of individuals reduced by 13% compared to the control group.

The use of feed supplements in the aquatic environment has certain difficulties in comparison with other econiches, in particular, the active components of feed may dissolve in water or washout if the aquaculture does not immediately consume it [102].

Use of commercial preparations. Probiotic strains of microorganisms, thanks to their wide spectrum of biological activity, are actively used in aquaculture; however, laboratory research data are insufficient for the use of them on an industrial scale. Therefore, the screening of probiotic strains for aquaculture is one of the most important stages of step-by-step fundamental scientific research, and their use is not limited to laboratory scales.

The method for administration and dosage form of probiotic preparations for aquaculture varies. Probiotic preparations for commercial use are available in the liquid or powder form. The use of lyophilized probiotic preparations has significant advantages during storage and transportation; however, they require certain conditions for the activation of microorganisms, in particular specific temperature, hydration degree, etc. [103]. After all, in order to perform a probiotic effect on aquaculture, the microorganisms contained in a preparation must survive during storage and in the GI tract conditions of aquatic species, remaining active and having a positive effect on individuals of various ages [104].

Farms use different methods for probitics administration: simultaneous use of several types of probiotics, use of probiotics in combination with prebiotics (food ingredients that selectively stimulate the growth and/or activity of microorganisms in the colon), addition of preparation to feed or water, administration of live strains of microorganisms or inactivated cultures, continuous (or in regular intervals) administration, as well as joint administration of probiotics with prebiotics (synbiotics) or plant products.

The use of probiotic preparations based on several strains is more effective than preparations containing a single strain or species of microorganisms [105]. The composition of *L. acidophilus* and *B. subtilis* strains improves antimicrobial activity against pathogenic microorganisms in Nile tilapia and hematocrit indicators in contrast to monocultures [31, 63, 75]. Also, the use of a feed supplement in combination with *L. lactis* and *B. plantarum* bacteria inhibits the development of *S. iniae* in Japanese flounder [106]. The positive effect was demonstrated with using monoculture of the *L. mesenteroides* strain or together with *E. faecalis* and *L. fermentum* in carp.

As shown by Yukhimenko L.N. et al. [102], the most effective feed supplements for the prevention/treatment of diseases of various etiologies in aquaculture are probiotics, prebiotics, and synbiotics. Synbiotics are drugs of a new generation, a rational combination of probiotics and prebiotics based on the principle of giving the probiont an advantage over competing endogenous populations of microorganisms with subsequent colonization the macroorganism's GI tract. Symbiotic use of *E. faecalis* strain together with oligosaccharide mannan promotes better feed conversion than prebiotics or probiotics alone [107].

The effectiveness of using live or inactivated probiotic microorganisms in aquaculture remains a controversial issue. It was established that the use oflive or freeze-dried cells of the probiotic strain *L. rhamnosus* JCM 1136 promotes an increase in the phagocytic activity and activation of the complement system in rainbow trout, in contrast to the use of thermally inactivated cells [108]. However, in the work of Irianto et al., the addition of heat-inactivated microorganisms improved the parameters of the innate immunity of fish [109].

The market presents commercial preparations based on strains of lactic acid bacteria for aquaculture (Table 2), which contain one or more live microorganisms used to improve the growth of hydrobionts. According to the literature, the industrial use of probiotics Cernivet LBC and Toyocerin in aquaculture contributed to the reduction of edwardsielloses, ensuring higher survival of individuals [110]. The use of the probiotic drug *PrimaLac* based on the composition of LAB strains contributed to the increase in the gonadosomatic index and the birth of babies [111]. Taoka et al. used compositions of the microorganisms *B. subtilis, L. acidophilus, C. butyricum,* and *S. cerevisiae* in growing tilapia and demonstrated an improvement in non-specific immune response indicators [45].

In addition to the abovementioned probiotic preparations, a number of products without the indicated composition of microorganisms are presented on the market of Ukraine. The probiotic preparation «Sansai Biorex Probiotics» «Bactoplus Lacto Health», which includes strains of lactic acid bacteria, is widely used for the prevention and control of infectious diseases, improving the immune status of aquaculture and restoring the normobiota of individuals. The

Table 2. **Commercial probiotics based on lactic acid bacteria for aquaculture**

Product	Company	Composition
Prosol	Prosol Chemicals	*B. longum, L. acidophilus, L. rhamnosus, L. salivarius, L. plantarum*
SYNSEA	™ FeedAd	*Lactobacillus* sp., *Bacillus*
Grobact	Tropical Biomarine System	*L. rhamosus, L. acidophillus, S. boulardii, B. coagulans, St. thermophilus, B. longum, B. bifidum*
Prolacto	Drug International	*L. acidophilus, B. bifidum, L. bulgaricus, L. fructooligosaccharides*
PrimaLac	Star-Labs	*L. acidophilus, L. casei E. faecium B. thermophilum*
Hydroyeast Aquaculture	Agranco Corp	*S. faecium, L. acidophilus, Yeast, Bifidobacterium* sp.
Biotix Plus	Matrix Biosciences	*Lactobacillus* sp.
Lact-Act	Geomarine Biotechnologies	*L. sporogens*
AquaStar	Biomin	*Pediococcus* sp., *Lactobacillus* sp., *Enterococcus* sp.
Aqua Culture	Arshine Feed	*L. plantarum, L. acidophilus, L. casei, L. faecalis, Clostridium butyricum*

preparation «Colombo FMC-50» is actively used to suppress the fungi (Saprolegnia) and white spot (Ichthyophthirius), which widely affect aquaculture. Preparation «Bioferm, probiotic for fish» is widely presented on Ukrainian market as a probiotic drug for aquaculture that stimulates the growth of fish and shrimps, suppresses the development of bacterial and viral pathogens. Food supplement for aquaculture feed «Bactoplus Food Spray PSB» based on microorganisms and a complex of vitamins with microelements stimulates the growth of fish, improves its health, and has an antioxidant activity.

Conclusions. This review of literature data indicates a significant positive effect of probiotic strains of lactic acid bacteria on aquaculture. The results of scientific research contribute to the active use of them for improvement of functional properties of macroorganisms. Due to the preventive use of probiotics as an ecological alternative to antibiotics, the productivity of aquaculture has been significantly improved.

Probiotic preparations based on lactic acid bacteria strains have significant advantages for stable cultivation of various types of fish, mollusks, shrimps, etc. through improving immunity, resistance to infectious diseases, and reducing the risk of mortality. In addition, microorganisms stimulate growth, have a positive effect on the fish productivity due to their effects on digestive enzymes activity and consumption of carbohydrates, fatty acids, and minerals. However, the «universal probiotic strain» cannot be used to obtain a good result in industrial conditions, it is therefore necessary to investigate the optimal conditions not only for survival and reproduction, but also for the effective action of LAB strains in the GI tract of hosts in a certain environment. The use of highly effective probiotic strains for target fish species in the aquatic environment requires further studies, as it is necessary to consider not only the harsh conditions of host's GI tract, but also the aquatic environment. The use of encapsulated LAB strains in alginate-gelatin microgels can significantly increase their viability and functional activity under such conditions.

Therefore, the use of probiotic strains of lactobacilli is a necessary prerequisite for increasing the volume of aquaculture cultivation and obtaining high-quality and safe products. This, in turn, is the key to sustainable aquaculture and meeting the growing global demand for environmentally friendly animal protein.

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ПРОБІОТИЧНІ ПРЕПАРАТИ НА ОСНОВІ
МОЛОЧНОКИСЛИХ БАКТЕРІЙ ДЛЯ АКВАКУЛЬТУРИ

Аквакультура забезпечує мільярди людей поживними речовинами: амінокислотами, вітамінами, мінералами та білками. Вирощування аквакультури комерційних видів швидко розвивається, займаючи вже близько третини світового виробництва риби, і є основною альтернативою зменшення чисельності диких популяцій, які не здатні задовольнити зростаючий продовольчий попит. До нормобіоти шлунково-кишкового тракту (ШКТ) аквакультури належать молочнокислі бактерії (МКБ) родів *Lactococcus, Lactobacillus, Enterococcus, Leuconostoc, Pediococcus, Streptococcus, Carnobacterium* та *Weissella.* Вони відіграють важливу роль, оскільки стимулюють синтез ферментів травлення, запобігають кишковим розладам, покращують імунну відповідь макроорганізму, підвищують бар'єрну здатність слизової оболонки в результаті колонізації ШКТ риб; активують стійкість до розвитку бактеріальних, вірусних та грибкових інфекцій за рахунок продукування широкого спектру антимікробних речовин (бактеріоцин, органічних кислот, пероксиду водню та ін.). Тому лактобактерії відносять до потенційних пробіотичних штамів для аквакультури. Сумісне використання МКБ з кормами підвищує їхню харчову цінність, оскільки мікроорганізми продукують широкий спектр ферментів травлення, що беруть активну участь у процесах травлення та розкладання корму, а також позитивно впливають на ріст особин та стимулюють їхню репродуктивну активність, що безперечно сприяє активному впровадженню комерційних пробіотиків на основі штамів МКБ. У результаті вирощування аквакультури виникає багато проблем, таких як щільність посадки аквакультури, інфекційні захворювання, надмірне використання антибіотиків, забруднення води, які можна вирішити шляхом використання МКБ з пробіотичними властивостями. Тому в огляді наведено сучасні літературні дані з використання штамів МКБ як пробіотичних препаратів для аквакультури, їхнього видового складу, впливу на організм хазяїна, механізму дії пробіотиків на аквакультуру та способів доставки до макроорганізму.

Ключові слова: *молочнокислі бактерії, аквакультура, пробіотичні штами, риба, пробіотики.*