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# A mini-review: Effect of probiotics on the growth and health of fish

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**Abstract**
The aquaculture sector industry is growing in order to meet the needs of animal protein at a price that is relatively affordable by various groups of people compared to other animal proteins. The development of the fisheries is influenced by the availability of quality fish free from disease attacks and good growth. Probiotics are good microbes that can suppress the growth of pathogenic microbes and can help accelerate fish growth. Various types of probiotics have been shown to increase the growth of various types of fish. In addition, various types of probiotics can also help prevent attacks of various types of pathogenic bacteria in fish. This article aims to explain various probiotics and the effect of probiotics on the growth and health of fish. Based on the above studies, various probiotics has the potential to be used as an alternative supplement for the Growth and Health of various types of fish.

**Keywords:** Disease, growth, immunostimulant, probiotics

## Introduction
The aquaculture sector industry is growing in order to meet the needs of animal protein at prices that are relatively affordable by various groups of people compared to other animal proteins [1]. Maintaining the quality of aquaculture products is influenced by several factors, including the availability of quality fish fry, mastered cultivation techniques, optimal feed requirements and appropriate environmental conditions and free from pathogen attack [2]. This condition is easy to control if it is in a closed cultivation system, but in an open cultivation system (Outdoor) it is rather difficult to control [3]. The main challenge in fish farming is the presence of pathogen attacks [4] and the availability of feed [5], this can weaken the fish's body defense system, if not addressed immediately can result in fish death [6], which is certainly very detrimental to the fishery, fishing industry players.

The attack of bacteria and viruses as the cause of death in fish can cause a decrease in production and is very economically detrimental [7]. The bacteria that often infect fish are *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Edwardsiella ictaluri* [8], *Edwardsiella tarda* [4]. The use of antibiotics in controlling disease in fish is highly recommended, but antibiotics have side effects, including causing bacteria to be resistant to these antibiotics, antibiotic residues can pollute the environment and accumulate in the fish's body, so that it will indirectly affect the human body that consumes them [1, 9]. Therefore, several countries have banned farmers from using antibiotics and switched to using alternative materials that are safer and more environmentally friendly [10].

Probiotics have been widely used in aquaculture and are used as a substitute for antibiotics for disease control in fish and shrimp. In addition, the use of probiotics in aquaculture can result in increased growth and feed digestibility [11]. The same thing was stated by [12], probiotics are a substitute for antibiotics and can suppress the growth of pathogens without harming the host and the environment. Probitotics can increase the body's resistance to pathogens [13, 14] and increase the activity of digestive enzymes that will affect fish growth. According to Dawood *et al.* (2018) [15] and RingØ (2020) [16] probiotics can increase the diversity of intestinal microflora, compete with harmful microorganisms, facilitate nutrient absorption through intestinal villi, and increase the natural (innate) immunity of the fish body. probiotics can be applied to improve the health and growth of fish in an environmentally friendly manner [17]. This article aims to describe the types of probiotics that have an effect on the growth and health of fish.

**Variety of probiotics in aquaculture**

Various bacteria have been used as probiotics for aquatic animals, including *Lactobacillus plantarum, Lactococcus lactis, Enterococcus faecium, Bacillus subtilis, and Saccharomyces cerevisiae* [18]. The bacterium *L. plantarum* (LP20) has recently been used as a probiotic in the feed industry, because it is non-toxic and significantly resistant to low pH conditions and is resistant to salt levels [19], and has beneficial effects on the host. [20] and also stable under processing conditions [21]. Inclusion of LP20 in tilapia diet can increase body resistance and antioxidant against toxicity and have anti-inflammatory effect on gills, liver, spleen, and intestinal tissue. In addition, tilapia is protected from ammonium chloride poisoning [22].

As according to Merrifield *et al*. (2010) [23] several types of probiotics used in aquaculture include *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus sakei*, *Lactobacillus delbrueckii*, *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Bacillus licheniformis*, *Aeromonas sobria*, *Carnocerevisia divergene*. However, according to El-Haroun *et al.* (2006) [24] the use of Bacillus strains as probiotics in feed is more profitable because it is more resistant during the pelletizing process (pelletization).

Some lactic acid bacteria (LAB) that are widely used as probiotics in fish are from the genus Vibrio, Bacillus, Pseudomonas and Roseobacter [25]. These probiotics can dominate the intestinal flora of fish if added to the feed, however the level of bacterial population in the digestive tract of fish is influenced by several factors including physiological conditions, nutrition and age, eating habits, season, salinity and stress levels of fish [26].

**Probiotics as antimicrobians**

Probiotic bacteria in inhibiting the growth of pathogenic bacteria in several ways, including lowering the pH and producing hydrogen peroxide. The Lactobacilli group of bacteria ferment lactose into lactic acid which can lower the pH to a level that cannot be tolerated by these pathogenic bacteria. Hydrogen peroxide produced can inhibit the growth of gram-negative bacteria [27]. According to McDonald *et al.* (2002) [28] and Klose *et al.* (2010) [29] lactic acid-producing bacteria from the genus Streptococcus and Lactobacillus can act as antibiotics. Meanwhile, according to Ringo *et al*. (2010) [25] the antagonistic effect of gut microbes with pathogenic bacteria occurs due to competition for nutrients and a place to attach to the intestine, as well as the formation of metabolites such as organic acids, hydrogen peroxide and also bacteriocins.

The results of research conducted by Rosidah *et al*. (2020) [30] showed that almost all isolates of lactic acid bacteria filtered from the intestines of carp (*Cyprinus carpio*) were able to inhibit the growth of pathogenic bacteria, namely *Aeromonas* sp. and *Vibrio* sp can be seen from the formation of a clear zone (inhibition zone) around the paper disc. Almost all isolates of lactic acid bacteria (15 isolates) could inhibit the growth of *Aeromonas* sp. with the resulting inhibition zone diameter ranging from 6.5 mm - 12.6 mm, the smallest inhibition zone was found in isolate 4, which was 2.0 mm. Lactic acid bacteria that can inhibit the growth of *Vibrio* sp. bacteria are only 10 isolates with a smaller diameter of the inhibition zone, which ranges from 2.30 mm - 5.27 mm. According to Pan *et al.* (2008) [31], bacteria with strong inhibitory ability will form a clear zone of more than 6 mm, moderate inhibition ability will form a clear zone of 3-6 mm, while a weak inhibition zone has the ability to form a clear zone of 0-3 mm. The resulting clear zone or inhibition zone is the effect of the antagonistic nature of microorganisms which will produce proteases, bacteriocins, lysozymes, hydrogen peroxide, formation of ammonia, diacetyl, and changes in pH values with the production of organic acids that can inhibit the growth of pathogenic microorganisms and other microorganisms [32, 33].

**Effect of probiotic on growth performance of fish**

Research on probiotics as a supplement to increase fish growth has been carried out. The role of probiotics in increasing fish growth is related to the ability of these probiotics to activate digestive enzymes [34, 35], thus helping the process of utilization and digestion of feed [36]. According to Kurniasih *et al*. (2013) [37] exogenous digestive enzymes have amylolytic activity (digest carbohydrates), proteolytic (digest protein), and lipolytic (digest fat). Several studies have proven that probiotics can increase the growth of carp (*Cyprinus carpio*) [38], tilapia (*Oreochromis niloticus*) [39], *Chanos chanos* [40] and *Labeo rohita* juveniles [41].

Probiotic bacteria isolated from the digestive tract of catfish were identified as *Lactobacillus* sp. which was applied orally to carp fry was able to improve the growth performance of carp. *Lactobacillus* sp. with a density of 1 x $10^8$ CFU/mL given for 30 days resulted in absolute growth, the highest daily growth and the lowest FCR, respectively 10.42±0.53, 2.98±0.10% and 1.31±0.08 with feed efficiency of 76.46±5.09%, while the seeds goldfish not given probiotics (control) resulted in lower absolute growth and daily growth and high FCR, respectively 6.25±0.24, 2.14±0.83% and 2.04±0.08 with feed efficiency of 48.95±2.05% [38].

Giving probiotics *Saccharomyces cerevisae* and *Bacillus subtilis* for 6 weeks can improve growth performance in tilapia. The weight gain of untreated tilapia (control) after 6 weeks was only 5.8±0.25 grams, tilapia fed with probiotic S. cerevisiae at a dose of 10g/kg of feed was 10.7±1.58 and those given a mixture of probiotics *S. cerevisiae* and *B. subtilis* with a dose of 1.5 g/kg feed of 12.3±1.45 grams. The value of feed conversion ratio (FCR) for each treatment was 5.8655, 3.0146 and 4.7379. Protein efficiency ratio (PER) for each treatment were 23,868, 46.44 and 49,517, respectively. Here, it can be seen that the increase in growth and the highest PER value was found in tilapia given the probiotic *S. cerevisiae* and *B. subtilis* at a dose of 1.5 g/kg feed, but the lowest FCR value was found in tilapia given the probiotic *S. cerevisiae* 10g/kg feed. However, tilapia treated with probiotics resulted in higher weight gain and PER and lower FCR compared to controls [39].

Milkfish fed commercial probiotics for 60 days showed a higher relative growth rate and lower FCR compared to controls. The commercial probiotics used contain *Saccharomyces cerevisiae, Aspergillus oryzae, Lactobacillus acidophilus, Bacillus subtilis, Rhodopseudomonas, Actinomycetes* and Nitrobacter bacteria where each content is > 1 x 1011 CFU. The average growth rate and FCR values of milkfish given probiotics were 1.958±0.02% and 1.321±0.030, respectively, while milkfish that were not given probiotics (control) the average growth rate and FCR were 1.573±0.02 and 1.581± 0.035. The efficiency of feed utilization for fish fed with probiotics was higher than the control, at 78.333 ± 0.745% and 63,000 ± 0.760%, respectively [40]. Research has been carried out by [41] on the growth of *Labeo rohita* fish using Commercial probiotic

'Ecotec' containing > 2 billion cfu freeze dried mixture of four different microbial strains, namely *Lactobacillus acidophilus*, LA-5, Bifidobacterium, BB-12, *Streptococcus thermophiles*, STY-31, and *Lactobacillus delbrueckii* ssp. Bulgarian, LBY-27. Probiotic was mixed with the basal diet in two different proportions: $8 \times 10^7$ CFU/g feed and $16 \times 10^7$ CFU/g feed. The results showed that fish fed probiotics for eight weeks resulted in greater weight gain compared to controls, respectively, 57.9 ± 2.5% ($8 \times 10^7$ CFU/g feed), 51.9 ± 1.5% ($16 \times 10^7$ CFU/g feed).) and 30.1 ± 2.2% (control). Likewise, the FCR value was lower than the control by 2.1 ± 0.3, 2.4 ± 0.4 and 4.1 ± 0.9, respectively. The selected LAB isolates of *P. pentosaceus* E2211 is potential for probiotics candidate in normal catfish and gnotobiotic catfish (*Clarias* sp.), that is indicated by the survival rate of Np and Gp treatment after challenge test, it was 88.46%, while K+ and G were only reached 53.84% and 65.38%. The performance of *P. pentosaceus* E2211 as probiotics was showed through its ability to maintain the survival rate, the specific growth rate, the total intestinal bacteria, and the immune response of catfish to *A. hydrophila* infection. The existence of normal microflora in association with *P. pentosaceus* E2211 in Np treatment showed the best performance of probiotic with 3.28%/ day of the specific growth rate and 1.78 of the feed conversion ratio. While the controls were 2.12%/day and 1.95 respectively [42]. The lower feed conversion ratio indicates that the feed absorption process given probiotic supplements is better than the control. The better the probiotics that are mixed into the feed, the greater the amount of protein and other nutrients that can be digested and used for growth, so that the FCR value can be lower. The low FCR value also indicates that the total cost required for fish production is also low.

**Effect of probiotic on health of fish**

In the field of aquaculture, the presence of probiotics is very beneficial, because it has an effect on fish health, where probiotic microbes can overcome disease attacks or reduce disease outbreaks. Probiotics can be used as an alternative for controlling diseases in fish caused by pathogenic microorganisms [43]. As according to Sadeghi *et al.* (2021) [44] and Yao *et al.* (2020) [45] probiotics have a beneficial effect on gut health and innate immunity, due to the live nature of probiotics that can compete with pathogenic microorganisms and colonize in the digestive tract. Multispecies can help increase specific or non-specific immune systems in fish, by inducing phagocytic cells, lysozymes and various cytokinins [46].

Research on probiotics as a supplement to improve fish health has been widely carried out. As the results of research conducted by Shabirah *et al.* (2019) [47] showed that goldfish that had been given probiotic lactic acid bacteria (LAB) had an increase in lymphocytes (part of leukocytes), where an increasing number of leukocytes during the challenge test indicated the fish body's defensive reaction against bacterial pathogens that enter the body (antigen) [48]. The probiotics used in this study were isolated from the intestines of carp, consisting of isolates CcB7, CcB8, and CcB15. The use of this probiotic through immersion for 24 hours and repeated within one week. The highest levels of lymphocytes were found in fish soaked in LAB isolates of CcB15, which was 82.00%, while fish that were not soaked in LAB (control) was only 75.67%. After being challenged with the bacteria *Aeromonas hydrophila*, carp given the probiotic CcB15 resulted in the highest survival, namely 83 ± 6.62%, while the control was only 33 ± 17.68%. This probiotic has also been used by Rosidah *et al.* (2020) [30] to prevent goldfish from being attacked by the *Edwardsiella tarda* bacteria, but from the results of his research, CcB7 isolate was the most effective isolate to prevent this bacterial attack. The LAB isolates CcB7 increased the levels of leukocytes, erythrocytes and hematocrit the highest by 18 ± 0.057%, 7 ± 0.077% and 0.26 ± 7.31%, respectively. Goldfish after The challenge test with the bacteria *E. tarda* produced mild clinical symptoms, an increase in monocyte and neutrophil by 20% and 62%, and caused a decline in the number of lymphocytes up to - 9% and survival by 80%. As according to Panigrahi *et al.* (2004) [49]. LAB probiotics can not only improve the immune system of fish, but can also improve water quality, thus affecting the survival of larvae and aquaculture in general.

The results of the study Insanie (2019) [50] to prevent carp from being attacked by A. hydrophila bacteria using Bacillus sp probiotics with different densities mixed into feed resulted in different survival. Probiotics were given for two weeks, the density of the probiotic Bacillus sp. used, ie. $10^6$ cfu/mL feeds, $10^8$ cfu/mL feeds and $10^{10}$ cfu/mL feeds. The results showed that Bacillus sp. into feed with a density of $10^8$ CFU/mL was most effective for increasing carp body resistance from the attack of A. hydrophila, and it was seen from carp survival of 89% and reduced clinical symptoms. only 61%. From the description above, it shows that probiotics can be used to increase the body's resistance to attack by pathogenic microbes. The type and dose of probiotics as well as the duration of probiotic administration significantly affect the immunomodulatory activity of probiotics. This is related to the content of secondary metabolites contained by probiotics to release various chemicals that can stimulate and induce the proliferation of immune cells, such as T and B lymphocytes, and macrophages. Probiotics are also bio-proteins containing live microbial cells that can optimize colonization and growth composition and intestinal microflora in animals and stimulate immune processes [51].

**Competing interests**

Author has declared that no competing interests exist.

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