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Original Article

# Isolation and characterization of lactic acid bacteria from the fish intestine for application as probiotics in young common carp *Cyprinus carpio* L. diet

**Jalal M. Al-Noor[1*], Salah M. Najim[1], Wael A. Al-Waely[2]**

[1]Department of Fisheries and Marine Resources, University of Basrah, Basrah, Iraq.
[2]Department of Food Science and Biotechnology, College of Agriculture, University of Basrah, Basrah, Iraq.

**Abstract:** The current study was conducted to investigate the possibility of using Lactic acid bacteria LAB isolated from the intestines of common carp, *Cyprinus carpio*, as dietary probiotics to enhance the nutrition and growth of common carp juveniles. The bacteria were cultured on MRS agar and their cultural and biochemical characteristics were confirmed. The isolated probiotic bacteria were used as dietary supplements at concentrations of $10^6$, $10^7$, and $10^8$ and their effect on the nutrition and growth of common carp fingerlings was evaluated. The results showed clear positive effects of adding the probiotic Lactobacilli in fish feed, with a relative growth increase from 105.26 to 178.14% with increasing probiotic concentration compared to the control. The feed conversion ratio also improved from 2.94 to 1.83 with increasing levels of probiotics compared to the control. In addition, all other indicators improved, including weight gain, specific growth rate, protein efficiency ratio, digestibility, and beneficial intestinal flora, with increasing probiotic concentration in the feed compared to the control, with a significant difference. It was concluded that LAB isolated from the intestines of common carp.can be effectively used as probiotics in common carp *C. carpio* fingerlings feed, with positive results.

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## Introduction

Fish culture, especially in space-limited systems such as floating cages and earthen ponds with high densities, faces problems such as crowding, competition, oxygen depletion, and deteriorating water quality, which in turn exposes fish to stress, inhibition of the immune system and growth, and the increase in the spread of disease infections (Beiwi and Al-Hisnawi, 2020). Therefore, to reduce stress, control disease outbreaks, and improve growth performance, aquafeeds were widely supplemented with a variety of additives to obtain high-quality diets (Singh et al., 2021). Many fish feed supplements are well-known for supporting growth rates, feed and protein conversion efficiency, improving diet digestibility, reducing mortality, and increasing disease resistance (Kord et al., 2021; Amit et al., 2022).

Probiotics and prebiotics are two classes of modern feed supplements that gain increasing significance for their safety and positive influence on general animal health (Xiong, 2018; Lund, 2021). Many probiotic species are applied effectively in aquaculture nutrition which contributed significantly to improving nutrient availability, feed efficiency, growth, and feed conversion rates (Shao et al., 2019; Watts et al., 2020), as well as enhancing the immune response, disease control and health promotion (Al-Hassani and Mustafa, 2022). The probiotic mechanism of action in the digestive system includes modifying the metabolism of pathological bacteria by changing their paths, and stimulating the body's cellular immunity and disease resistance, by occupying receptors, which leads to the inhibition of colonization by harmful organisms (Goran et al., 2017; Chizhayeva et al., 2022). Probiotics could be consisting of a single species or mixture of live microorganisms that positively affect the health of

*Correspondence: Jalal M. Al-Noor
E-mail: jalal.essa@uobasrah.edu.iq

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the host by improving the characteristics of the original normal gut microbiota with increasing the numbers of beneficial and decreasing the numbers of pathogenic species (Rhema and Al-Noor, 2022). Many species of gram-positive and Gram-negative bacteria and other microorganisms such as yeast, Enterobacteriaceae, and unicellular algae are recently considered as probiotics (Suman et al., 2016). A number of lactic acid bacteria (LAB) especially species of the genus *Lactobacillus* are widely used as probiotics, and species of other genera like *Bacillus*, *Enterococcus*, *Streptococcus*, and *Bifidobacterium* (Watts et al., 2020). Therefore, this study was performed to isolate and characterize LAB from the *Cyprinus carpio* L. intestine and investigate their utilization as probiotics in diets for common carp juveniles.

## Materials and methods

**Isolation of lactic acid bacteria:** Five common carp were obtained from the local markets of Basrah city, with an average weight of 1938 g and a total length of 38.88 cm. Fish were dissected and the first third of their intestines were taken and cleaned, gut contents were removed aseptically and washed several times with a sterile washing solution to remove the rest of the microorganisms loosely associated with the intestine. The clean-washed intestines were cut with sterile scissors into small pieces and placed in a sterile flask containing the sterile dilution solution. One milliliter of the solution containing the sample was transferred to a test tube containing 9 ml of physiological solution to prepare a series of decimal dilutions up to $10^8$. The last three decimal dilutions of $10^6$, $10^7$ and $10^8$ were cultured, using 1 ml from each dilution, and the samples were cultured using the pour plate method on MRS Agar according to Awan and Rahman (2005). Then, the plates were incubated in an anaerobic incubator at a temperature of 37°C for 48 hours.

After the end of the incubation time, the plates were removed from the incubator, the growing colonies were distinguished morphologically, and parts were transferred to new Petri dishes containing MRS Agar for obtaining pure colonies. The growing *Lactobacillus* colonies were isolated and grown on MRS Broth and incubated at 37°C for 48 days. Then, the bacterial cells were harvested by centrifugation at 2000 rpm. The density of the growing bacterial cells was determined using a spectrophotometer according to MacFarland's method.

Isolated bacteria were used in diets at rates of $10^6$, $10^7$, $10^8$ and $10^9$ cfu/g, as follows: Liquid cultures were prepared by growing bacteria at the age of 48 hours by anaerobic incubation at 37°C in a nutrient broth manufactured by Fluka© (Japan) and prepared according to the company's instructions. Serial dilutions of the liquid bacterial culture up to $10^9$ were prepared using sterile normal saline (physiological solution) and 9 ml was distributed into each glass test tube that has been autoclaved. The absorption spectrum for each bacterial culture was determined using a spectrophotometer at 650 nm according to Gerhardt (1993). Bacterial cell suspensions were added to the diets by spraying with a hand sprayer and kept to air dry before being stored in the refrigerator at 4°C inside tightly closed plastic bags until use.

### Diagnostic tests

**Culture and microscopic tests:** Colonies with a creamy color and convex shape surrounded by a transparent halo were transferred using a loop to test tubes containing MRS broth and incubated at 37°C for 24 hours, then stained with gram stain to examine morphology based on criteria of single, bilateral, sequential, gram-positive and non-spore-forming bacilli. The locomotion test was also carried out using the hanging drop method (Harley and Prescott, 2002). Positively diagnosed colonies were preserved for further biochemical tests.

**Biochemical tests:** The biochemical tests were conducted for the diagnosis of lactobacilli, including catalase, gelatinolytic activity, indole production, ammonia production from arginine, carbohydrate oxidation/fermentation, nitrate reduction, and citrate consumption.

**Experimental fish and husbandry system:** For the feeding experiment, the juvenile common carp were

Table 1. Composition (%) and added bacteria for different experimental diets.

| Feedstuff | Control | T1 | T2 | T3 |
|---|---|---|---|---|
| Fish meal | 23 | 23 | 23 | 23 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Corn | 15 | 15 | 15 | 15 |
| Wheat bran | 20 | 20 | 20 | 20 |
| Wheat flour | 18 | 18 | 18 | 18 |
| Premix | 2 | 2 | 2 | 2 |
| Vegetable oil | 2 | 2 | 2 | 2 |
| Bacteria cfu/g | 0 | $10^6$ | $10^7$ | $10^8$ |

used with an average weight of 15.34 g, which were obtained from the aquaculture unit of the College of Agriculture, University of Basrah. In the laboratory, fish were disinfected using 3% saline solution for one minute to get rid of possible attached bacteria and parasites (Noga, 1996). Then, fish were raised in 30-liter glass aquariums previously sterilized with 200 ppm sodium hypochlorite solution for one hour (Herwing et al., 1979). Aquariums were equipped with plastic mesh covering to prevent fish escape, compact aeration-filtration units, and submerged heaters to maintain water temperature. The experiment was designed with four treatments each with 3 replicates (in a total of 12 aquaria). Initially, fish were randomly assigned to the different treatments at a rate of 10 fish/ aquarium and acclimated to laboratory settings for ten days, during which they were fed a standard diet (Table 1, control one).

**Diet preparation:** After determining the proportions of the different feedstuffs (Table 1), used feedstuffs were ground to the appropriate size, mixed in the calculated proportions to homogenize, then 40% boiling water was added to the mixture. After suitable mixing, the temperature of the mixture was raised to 80°C and left to cool. Vitamin and minerals premix was added immediately before the diet's final formation. Four experimental diets were formulated with added bacteria as follows: T1, with $10^6$ added bacteria, T2, with $10^7$ added bacteria, T3, with $10^8$ added bacteria and C, control (without added bacteria). Experimental diets were formulated into pellets using a meat grinder and then left to dry at ambient temperature for 48 hours with continuous stirring to ensure complete drying before cool storage in plastic containers until use.

**Feeding experiment**

**Fish growth:** During the experiment, fish were fed experimental diets at a daily rate 3% of the body weight divided into two meals (at 8-9 am and 1-2 pm). Fish were weighed biweekly to adjust diet and approximately 25% of aquarium water was changed daily while siphoning uneaten feed and wastes. Fish growth parameters, including total weight gain (TWG) and daily weight gain (DWG) were calculated according to Sevier et al. (2000) using the formula of TWG (g/fish) = Final weight – Initial weight, and DWG (g/fish/day) = TWG / time (day), respectively. Relative (RGR) and specific growth (SGR) rates were calculated based on Jobling (1993) using the formula of RGR (%) = TWG / Initial wt. X 100, and SGR (%/day) = (ln final wt. – ln Initial wt.)/ time (day) X 100. Furthermore, Food conversion ratio (FCR), protein intake (PI), and protein efficiency ratio (PER) were calculated according to Tacon (1990) using the formula of FCR = Consumed feed (g.) / TWG (g.), PI (g./fish) = Consumed feed (g.) x Feed protein content (%), and PER (%) = TWG / PI.

**Feed apparent digestibility:** To measure total (TADC) and nutrient (NADC) apparent digestibility coefficients, an indirect method described by Talbot (1985) was applied using chromium oxide $Cr_2O_3$ as a marker. Indicator content in experimental diets and collected fish feces was assessed by measuring absorbance spectrophotometrically at 350 nm.

TADC (%) = 100 – [100 x (% marker in feed) / (%marker in feces)

NADC = 100 – [100 x {(% marker in feed) /

(%marker in feces)}/ {(% marker in feces) / (%marker in feed)}]

**Microbiological examination of the fish intestine:** The microbial numbers were estimated by preparing decimal dilutions, taking 1 gr of the studied fish intestines, adding 9 ml of 0.1% sterilized peptone water solution, and mixing well to prepare the first dilution of $10^{-1}$. From it, the rest of the decimal dilutions were prepared in sterilized test tubes, each containing 9 ml of the dilution solution to obtain suitable dilutions of the samples. Then, the Pour plate method was used for all microbiological tests, by transferring 1 ml from each dilution to empty and sterilized Petri dishes and adding the nutrient medium at a temperature of 45°C, mixing the sample with the culture medium in the dishes thoroughly, and gently. The dishes were left until solidification, then placed upside down in the incubator at a temperature of 35°C for 24-48 hours. The numbers of growing microorganisms were expressed in colony-forming units (CFU/g), and the colony count was multiplied by the inverse of the dilution. Nutrient Agar was used for the total bacteria count, and MRS Agar was used for counting Lactic acid bacteria, according to the method described by Andrews (1992).

## Results and discussion

**Isolation of *Lactobacillus* bacteria:** Several studies have focused on isolating *Lactobacillus* bacteria from fish sources and marine animals to discover the better potential of LAB that could be implemented in different industries such as food and beverages, pharmaceutical, aquaculture, nutraceutical, and medical (Rialita et al., 2019; Lambuk et al., 2022). Al-Faragi and Alsaphar (2012) and Ekundayo (2014) reported several LAB isolates from shrimps and fish including the intestines of common carp. Ismael (2022) also isolated probiotic bacteria from Nile tilapia, *Oreochromis niloticus* showing their inhibitory effect against pathogenic bacteria, while also improving blood and immune parameters and promoting fish growth. Kafi et al. (2022) pointed out that lactic acid bacteria isolated from the intestines of carp have potential use in aquaculture nutrition where it contributed significantly to improving the tolerance of farmed fish to salinity and temperature. Generally, fish gut and muscle naturally contain various LAB species, making them a promising candidate as probiotics for aquaculture. Among the LAB genera commonly isolated from fish are *Lactobacillus, Lactococcus, Enterococcus, Leuconostoc, Carnobacterium,* and *Vagococcus* (Ringo et al., 2018).

**Cultural and microscopic characteristics:** The isolation of colonies was based on the formation of a transparent halo around the developing colonies. The results showed that isolated colonies on MRS Agar are *Lactobacillus* bacteria, as this medium is specifically used for their isolation. MRS medium contains acetate, which serves as a selective agent for *Lactobacillus* growth (Hayek et al., 2019). On the surface of MRS agar, the developing colonies exhibited standard specifications, characterized by a creamy color with a smooth edge. Most of them were non-glossy in appearance, some slightly convex and rough, and circular in shape. These characteristics were previously attributed to colonies formed by the genus *Lactobacillus* (Gupta et al., 2018). Microscopic examination revealed that the colonies have varying shapes, some were single, some paired, and others formed long or short chains. The colonies were gram-positive, non-sporulating, and non-motile (Soni et al., 2021). The bacterial isolate's specifications matched well with the results of Kumaree et al. (2015) who isolated LAB from the digestive tract of catfish. The results of the current study similarly coincided with those of Ekundayo (2014) and Salas-Leiva et al. (2017) who isolated LAB from fish intestines and pointed out that the shape of the rod is the distinguishing feature of *Lactobacillus* from other genera of LAB because it is the only genus that has this shape, while the rest are spherical.

**Biochemical characteristics:** Upon conducting biochemical tests on the isolated *Lactobacillus* bacteria, all isolates were not catalase-producing, non-gelatinase-producing, not utilizing citrate,

Table 2. Morphological and biochemical characterization of *Lactobacillus* isolated from *Cyprinus carpio* intestine.

| | Test | Results |
|---|---|---|
| 1 | Morphology | Single, paired or short chains |
| 2 | Growth conditions | Facultative aerobic |
| 3 | Motility | - |
| 4 | Gram stain | + |
| 5 | Catalase | - |
| 6 | Indole | - |
| 7 | Gelatinase | - |
| 8 | OF glucose | + |
| 9 | Arginine deiminase | - |
| 10 | Nitrate reduction | - |
| 11 | Citrate | - |

Table 3. Feeding and growth parameters of *Cyprinus carpio* in growth experiment.

| Treatment | Control | T1 | T2 | T3 |
|---|---|---|---|---|
| Initial weight, g. | 132.22±3.42[a] | 135.51±4.74[a] | 133.51±3.64[a] | 138.82±3.52[a] |
| Final weight, g. | 271.80±13.44[c] | 302.22±13.72[b] | 321.16±12.35[b] | 386.12±16.22[a] |
| Weight gain, g. | 139.58±19.22[c] | 166.71±17.44[b] | 187.70±16.43[b] | 247.30±21.53[a] |
| SGR, %/day | 4.55±0.28[c] | 4.80±0.11[b] | 4.96±0.22[b] | 5.30±0.21[a] |
| RGR, % | 105.26±15.52[c] | 123.02±9.83[b] | 140.64±13.87[b] | 178.14±18.41[a] |
| FCR | 2.94±0.98[d] | 2.24±0.58[c] | 2.38±0.21[b] | 1.83±0.16[a] |
| PER | 1.02±0.13[d] | 1.25±0.23[c] | 1.39±0.11[b] | 1.58±0.13[a] |

indole-negative, and capable of fermenting glucose (Table 2). These findings are consistent with previous studies such as Ekundayo (2014), Kumaree et al. (2015), and Bennani et al. (2017). Additionally, the characteristics of the isolates are in line with those reported by Majeed et al., (2017), Bintsis (2018), and Hayek et al. (2019) who separately indicated that the genus *Lactobacillus* can be differentiated from other LAB genera by its rod-shaped morphology.

**Fish growth experiment:** Table 3 shows the initial and final weights, total weight gain, daily growth rate, specific growth rate, feed conversion ratio, and protein efficiency ratio for the experimental fish. The results indicate that the highest values for final weight gain, total weight gain, daily growth rate, specific growth rate, and relative growth rate were recorded in the T3 diet ($10^8$ probiotic addition), where they reached 386.12 g, 247.30 g, 3.29 g/day, 5.30%/day, and 178.14%, respectively. The lowest values were recorded in the control diet, where they reached 271.80 g, 139.58 g, 1.86 g/day, 4.55 %/day, and 105.26%, respectively.

The results also showed that adding lactic acid bacteria to T1, T2, and T3 diets resulted in better growth compared to the control diet. The T3 diet outperformed all other diets in growth parameters, indicating the success of using probiotics in fish feeding for all treatments, but the T3 diet was the best with a significant difference ($P$<0.05) compared to other treatments.

Based on the results, the best feed conversion ratio was achieved in the T3 diet, reaching 1.83 with a significant difference ($P$<0.05) compared to the other treatments, which had feed conversion ratios of 2.94, 2.24, and 2.38 for the T0, T1, and T2 diets, respectively. The results showed significant differences ($P$<0.05) in feed conversion ratio among the treatments. The results also showed that the highest protein efficiency ratio was recorded in the T3 diet, reaching 1.58, while the lowest ratio of 1.02 was recorded in the control group. The remaining ratios were 1.25 and 1.39 for the T1 and T2 diets, respectively. The results showed significant differences ($P$<0.05) among all treatments.

Fish growth characteristics are very important practical criteria widely used to evaluate the quality of various feed and feed additives as they represent the final result relied upon by culturists in field settings (Rombenso et al., 2022). The results indicated that all targeted probiotic treatments significantly outperformed the control treatment. The reason may be attributed to the fact that *Lactobacillus* probiotics used as a feed additive contributes to an increase in growth indices and utilization of food, in addition to its role in the production of secondary metabolism, which produces substances that act as antibiotics and enhance the immune response (Azevedo et al., 2015; Nieto-Domínguez et al., 2017). The probiotic's effects in activating the immune system, reduce stress conditions, increasing feed intake, and consequently increasing fish growth rates. The probiotic's activity may also be attributed to its role in increasing growth performance as seen by the increase in the various relative and specific growth rates and weight gain (Wuertz et al., 2021). In addition, the significant superiority in all feeding and growth parameters of fish fed with the T3 diet (with a ratio of $10^8$ of probiotic additive) could be attributed to the increased counts of *Lactobacillus* bacteria and their positive effects on the digestive system and the different digestion and absorption processes (Amit et al., 2021).

The results indicate a superiority in specific growth rate and feed conversion efficiency in the probiotic groups compared to the control. This finding is consistent with the results of Rane and Markad (2015) who used the probiotic *Lactobacillus* spp. as a dietary supplement for Zebrafish, *Danio rerio*. The results also agree with Adineh et al. (2013) who achieved higher specific growth rates for Rainbow trout, *Oncorhynchus mykiss*, using the same probiotics and more recently *Xia et al. (2020)* reported that adding probiotics to the diet of Nile tilapia improves growth parameters and health and diseases resistance. The possible reason for these results is that high concentrations of probiotics contain large amounts of active substances, in addition to their role in providing fermentable substances that change the intestinal microbiota composition and lead to increased beneficial bacteria (Djauhari et al., 2017).

As observed from FCR results, there was an improvement which could be ascribed to the added probiotics and their role in enhancing the utilization of food materials, which is in turn a reflection of the improved function of the intestines through the development of beneficial microorganisms and their rapid adherence to the surface of the mucosal layer, which is suitable for their growth and multiplication, where they act to secrete digestive enzymes and fight pathogenic species (Amit et al., 2022). The results were consistent with those of Al-Asha'ab et al. (2014) pointed out that the use of probiotics in feeding common carp led to improvements in growth standards, increased feed conversion ratios, and better blood characteristics compared to the control treatment. The current results also agreed with Xia et al. (2020) who demonstrated that the use of probiotics containing *Bacillus* bacteria in fish feeding led to an increase in the feed conversion rate and improvement in the nutritional value and feed palatability. In addition, Feng et al. (2022) recently confirmed that adding probiotics in carp farming resulted in an increase in all growth measures, and improvement in feed conversion ratio with a significant variation from other experimental treatments. This may be due to the effects of beneficial bacteria in the digestive tract aid in digesting complex carbohydrates, proteins, and fats, increase the digestion coefficient and increase the absorption of nutrients (Guerreiro et al., 2018) resulting in the conversion of complex food materials into simpler ones more efficiently and increasing the production of volatile short-chain fatty acids, thus improving feed conversion efficiency (Djauhari et al., 2017; Nimalan et al., 2023). Qaddoori et al. (2023) previously mentioned a similar positive effect of using probiotics in feeding common carp. Amie et al. (2022) and Maniat et al. (2023) also found improvement in the feed conversion ratio, feed efficiency, immune response, and growth performance parameters for common carp fed probiotics for 8 weeks. A significant improvement was observed in blood characteristics such as hemoglobin levels, red and white blood cell counts, and total blood volume, as well as a

Table 4. Apparent digestibility coefficient of fish feed components.

| Feed component | ADC | | | |
|---|---|---|---|---|
| | Control | T1 | T2 | T3 |
| Protein | 88.26±0.02[d] | 90.11±0.11[c] | 93.24±0.27[b] | 95.72±0.02[a] |
| Lipid | 87.64±0.04[d] | 88.73±0.04[c] | 90.34±0.02[b] | 91.87±0.07[a] |
| Carbohydrates | 83.44±0.011[d] | 86.62±0.06[c] | 87.43±0.06[b] | 91.16±0.09[a] |

significant increase in total protein and globulin values, and albumin ratio, indicating an overall improvement in non-specific immune indicators.

The results showed the apparent digestibility coefficients of the nutritional components in experimental feeds containing different doses of LAB and fed to common carp during this study (Table 4). The results indicated significant differences ($P$<0.05) between the four treatments. The highest value for protein digestibility coefficient was recorded for treatment T3 (95.72) followed by T2 (93.24) while the digestibility coefficient values for protein in T1 varied and decreased in the control. As for the lipid digestibility coefficient, it increased with the increase in added LAB counts and was 88.73 in T1 but increased to 90.34 and 91.87 in T2 and T3, respectively compared to the control (87.64).

For carbohydrate digestion, the results showed significant differences between treatments ($P$<0.05), where the value for the control diet was 83.44, while it was increased to 86.62 in T1 (containing $10^6$ addition rate) and increased with the addition rate until it reached 87.43 and 91.16 in T2 and T3, respectively. This indicated that the best digestibility of nutrients was clearly observed with the addition of LAB to the diet. This can be attributed to the role of probiotics in increasing the digestibility and breakdown of undigested feed materials in the intestines through the production of various digestive enzymes which aid in hydrolysis of carbohydrates, proteins, and fats and simultaneously increasing their absorption. Additionally, they also help prevent digestive inhibitors in food, leading to an increase in the digestibility coefficients compared to the control (Taufik et al., 2019; Qaddoori et al., 2022). The superiority in the protein digestion coefficient is attributed to the role of beneficial microorganisms in the probiotic, as LAB are known to secrete some enzymes that break down the various feed components, increase their digestion coefficient and absorption, and thus increase their bioavailability. This leads to an increase in growth and protein efficiency ratio as a consequence of increased diet apparent digestibility especially protein digestion (Wuretz et al., 2021). The improved growth resulting from the use of LAB was previously explained by the improvement of the intestinal microbial balance, which leads to an increase in the digestion coefficient and high efficiency in absorption, an increase in enzyme activity, and the breakdown of high molecular weight proteins into smaller molecular weight amino acids and peptides (Guerreiro et al., 2018; Taufik et al., 2019).

Probiotics help in the digestion of carbohydrates by producing digestive enzymes, preventing intestinal disorders, and inhibit anti-digestion factors in food, and producing vitamins, especially vitamin B12, which is important for increased nutrient utilization (Assan et al., 2022). Assan et al. (2022) also indicated that the use of probiotics in fish nutrition could lead to stimulated activity of intestinal membranes and villi to secrete enzymes, thus improving growth performance and nutrient utilization and the improvement in nutrient digestion was possibly due to an increase in the digestion of proteins, fats, and carbohydrates in diets. Nimalan et al. (2023) investigated the potential of lactic acid bacteria in preventing soybean meal-induced enteritis in Atlantic salmon when fed for 39 days and demonstrated the effectiveness of this bacteria in reducing the width of the mucous layer and thus improving digestibility and survival rates. Additionally, Magouz et al. (2023) observed an improvement in the digestibility of Nile tilapia fed a balanced mixture of feed additives containing lactic

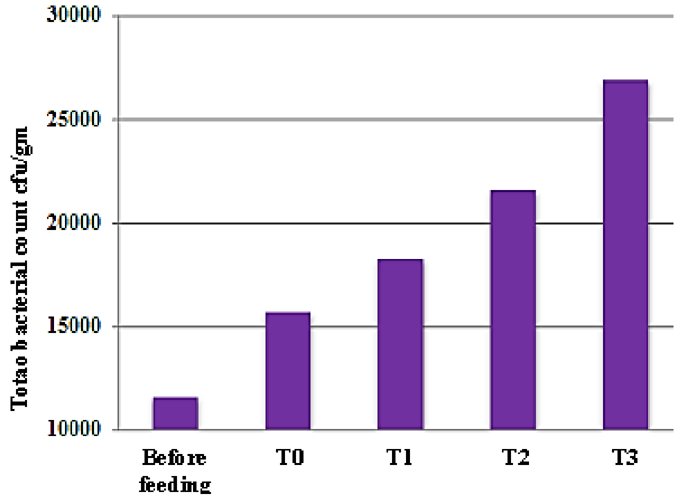


Figure 1. Total bacterial counts into intestines of *Cyprinus carpio* fed different LAB doses.

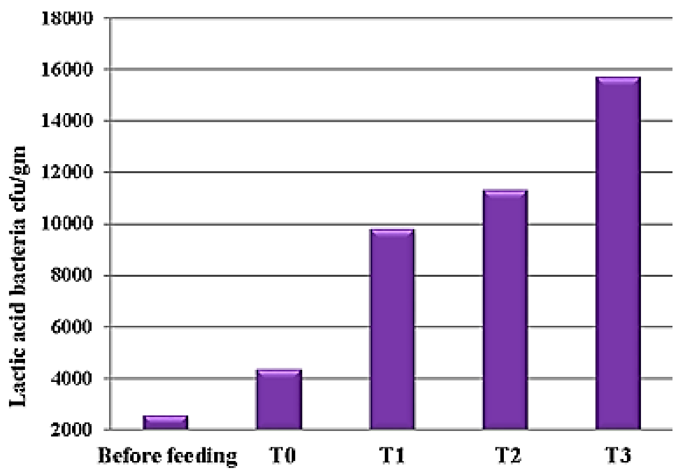


Figure 2. LAB counts into intestines of *Cyprinus carpio* fed different LAB doses.

acid bacteria with an increase in the activity of amylase, protease, and lipase enzymes which reflected in an increase in the density of intestinal villi, leading to improved growth and overall fish health.

**Microbiological tests:** The results demonstrated the microbial tests (intestinal flora) of fish fed on varying levels of LAB during this study (Figs. 1, 2). A clear variation was observed in the total number of bacteria before and after feeding. The highest values for the total number of bacteria were observed in T3, reaching $269\times10^2$ cfu/g (Fig. 1). Moreover, all experimental feeds gave varying numbers, with rates of $216\times10^2$ and $183\times10^2$ cfu/g for T2 and T1, respectively. The control diet had lower rates of $157\times10^2$ cfu/g compared to those recorded before feeding, which was $116\times10^2$. The average counts of LAB in the common carp intestines in the four treatments were $26\times10^2$, $44\times10^2$, $98\times10^2$, $113\times10^2$, and $157\times10^2$ cfu/g for fish before feeding, control, T1, T2, and T3, respectively.

Evaluating the impact of feeding fish with LAB diets on fish gut microbiota is essential for a comprehensive assessment of current aquaculture feeding strategies, as the composition of intestinal microflora has a significant impact on host health and is influenced by the diet (Cláudia et al. 2021). Some previous studies have indicated the role of the probiotic *Lactobacillus* in increasing fish appetite, enhancing digestive enzyme activity, improving feed conversion ratio, and improving the microbial environment of the digestive tract by promoting the growth of beneficial bacteria and eliminating harmful bacteria by preventing them from adhering to the intestinal epithelial cells which consequently enhances the immune response and controls bacterial diseases (Iman et al., 2013; Gupta et al., 2014). The findings of the current study also support Ringo (2020) and Al-Janabi et al. (2022) results, which addressed incorporating probiotics into fish feed to boost the immune response and bolster fish's ability to resist diseases by augmenting beneficial microorganisms in the gut. The results were consistent with the findings of Dhanaraj et al. (2010) who indicated a positive effect of *Lactobacillus* in increasing beneficial bacteria in fish intestines and preventing harmful bacteria from proliferating by spreading within the first line of defense inside GIT which is the mucus layer that releases microorganisms and is directly connected to epithelial cells, hindering harmful bacteria from adhering.

Another supporting evidence was provided also by Guerreiro et al. (2017) who found an improvement in fish health and increased growth rates for fish fed with probiotics and attributed it to the enhanced utilization of dietary components due to the increased effectiveness of the protease and amylase enzymes in the fish's intestinal content as showed also by previous studies (Anguiano et al., 2013; Azevedo et al., 2015). Alternatively, this may be attributed to the nature of the dietary probiotics, which work on the principle of competitive exclusion and inhibits the growth of pathogenic microorganisms in the digestive system by producing inhibitory substances or competing for food and space. Additionally, the presence of LAB as probiotics increases the effectiveness of epithelial cells and enzymes and enhances the immune response (Ismael, 2022). The results were consistent with the findings of Amit et al. (2022), where an increase in the number of lactic acid bacteria was detected in the gastrointestinal tract of fish groups fed with *L. plantarum* as a probiotic, enhanced immunity and growth. Generally, the complex and vast ecosystem of the intestinal microflora has a symbiotic relationship with its host, whether human or animal, and serves as a vital barrier against harmful microorganisms and toxins (Xia et al., 2019) and its supporting probiotics which reportedly boost the immune system will act collectively as harmonious elements in safeguarding the overall fish health by defending against pathogenic microorganisms (Meng et al., 2019).

In conclusion, the fish intestine could be a good and valuable source of LAB which may act as beneficial probiotics in fish nutrition. Isolation, characterization, and culture of this bacterial genus are proved feasible to be used later as a dietary additive. As an intrinsic bacterial genus, it is expected to gain more acceptability in future applications in aquaculture nutrition and technology.

## References

Adineh H., Jamarion H., Shandi J., Alizadeh M. (2013). Effect of *Bacillus* spp. probiotic on growth and feed performance of rainbow trout *Oncorhynchus mykiss* larvae. Bulgarian Journal of Veterinary Medicine, 16(1): 29-36.

Al-Hassani S.T., Mustafa S.A. (2022). Efficiency of symbiotic as feed additives on growth performance, survival rate and health status in *Common carp* challenged with *Saprolegnia* spp. Iraqi Journal of Agricultural Sciences, 53(2): 397-405.

Al-Asha'ab M.H., Mohammad S.D., Al-fathly M.K., Neamah Y.J. (2014). Effect of Using Probiotics with Prebiotics in Growth Indicia and Some Physiological Characters for Fingerlings Common Carp Cyprinus carpio L., Journal of Biotechnology Research Center, 8(2): 44-50.

Al-Dubakel A.Y., Al-Hamadany Q.H., Mohamed A.A. (2015). Effect of local probiotic (Iraqi probiotic) on the growth of common carp *Cyprinus carpio* L. youngs. Journal of Basrah Researches (Sciences), 41(B.3): 57-69.

Al-Faragi J.K.H., Alsaphar S.A.A. (2012). Isolation and identification of *Bacillus subtilus* as probiotic) from intestinal microflora of common carp *Cyprinus carpio* L. Proceeding of the Eleventh Veterinary Scientific Conference, 355-361.

Al-Janabi M.F., Al-Noor J.M., Al-Dubakel A.Y. (2022). Evaluation of Thepax and Bio Boost for stimulating microorganisms in the intestines of common carp *Cyprinus carpio.* Bionatura, 1-13.

Al-Janabi M.F., Al-Noor J.M., Al-Dubakel A.Y. (2021). Evaluation of theca and endo Vit. C as growth promoters for common carp *Cyprinus Carpio*. Natural Volatiles and Essential Oils, 8(6): 1976-1992.

Amit P.A., Khairnar S.O., Tyagi A. (2021). Effect of dietary supplementation of probiotic bacteria *Lactobacillus plantarum* on growth and proximate composition of *Cyprinus carpio* Fingerlings. National Academy Science Letters, 44(6): 495-502.

Amit P.A., Tyagi A., Khairnar S.O. (2022). Oral feed-based administration of Lactobacillus plantarum enhances growth, haematological and immunological responses in *Cyprinus carpio*. Emerging Animal Species, 3: 1-9.

Andrews W. (1992). Manual of food quality control. 1. Microbiological analysis. FAO Food and Nutrition Paper, 14(4 Revis 1): 1-338.

Anguiano M., Pohlenz C.A.B., Gatlin D.M. (2013). The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*). British Journal of Nutrition, 109: 623-629.

Assan D., Kofi F., Kuebutornye A., Hlordzi V., Chen H., Mraz J., Mustapha U.F., Abarike E.D. (2022). Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and pros-pects: A mini-review. Comparative Biochemistry and Physiology, B 257: 110653.

Awan J.A., Rahman S.U. (2005). Microbiology Manual. Unitech Communications, Faisalabad. pp: 49-51.

Azevedo R.V., Filho J.C.F., Cardoso L.D.C., Mattos D.C.M., Júnior M.V.V., Andrade D.R. (2015). Economic evaluation of prebiotics, probiotics and symbiotic in juvenile Nile tilapia. Revista Ciencia Agronomica, 46(1): 72-79.

Beiwi D.A., Al-Hisnawi A. (2020). Effect of Bacillus subtilis as probiotic on intestinal microbiota and growth performance of common carp (*Cyprinus carpio*). In AIP Conference Proceedings. AIP Publishing LLC, 2290(1): 030004.

Bennani S., Mchiouer K., Rokni Y., Meziane M. (2017). Characterization and Identification of *Lactic Acid Bacteria* Isolated from Morrocan Raw Cow's Milk. Journal of Materials and Environmental Sciences, 8(S): 4934-4944.

Bintsis T. (2018). *Lactic Acid Bacteria*: Their Applications in Foods. Journal of Bacteriology and Mycology, 6(2): 89-94.

Boulares M., Mejri L., Hassouna M. (2011). Study of the microbial ecology of wild and aquacultured Tunisian Fresh Fish. Journal of Food Protection, 74(10): 1762-1768.

Chizhayeva A., Amangeldi, A., Oleinikova Y., Alybaeva A., Sadanov A. (2022). Lactic acid bacteria as probiotics in sustainable development of aquaculture. Aquatic Living Resources, 35(10): 1-17.

Cláudia R., Serra A., Enes O.P., Tavares F. (2021). Gut microbiota dynamics in carnivorous European seabass (*Dicentrarchus labrax*) fed plant-based diets. Scientific Reports, 11: 447.

Dhanaraj M., Haniffa M.A., Arun S., Singh V., Jesu A., Ramakrishanan C., Seetharaman S., Arthimangu R. (2010). Effect of probiotic on growth performance of Kio carp (*Cyprinus carpio*). Journal of Applied Aquaculture, 22: 202-209.

Djauhari R., Widanari S., Supraydi M.A., Zairnijr M. (2017). Growth performance and health status of common carp (*Cyprinus carpio*) supplemented with prebiotic from sweet potato (*Ipomoea batatas* L.) extract. Pakistan Journal of Nutrition, 16(3): 155-163.

Ekundayo F.O. (2014). Isolation and identification of lactic acid bacteria from rhizosphere soils of three fruit trees, fish and ogi. International Journal of Current Microbiology and Applied Sciences, 3(3): 991-998.

Feng J., Liu S., Zhu C.L Cai Z.L Cui W.L., Chang X., Nie G. (2022). The effects of dietary *Lactococcus spp*. on growth performance, glucose absorption and metabolism of common carp, *Cyprinus carpio* L. Aquaculture, 546: 737394

Gerhardt P. (1993). Manual of Methods for General Bacteriology, 1st ed. American Society for Microbiology, Washington D.C. pp: 303-304.

Goran S.M.A., Omar S.S., Anwer A.Y. (2017). Assessment of yeast as a dietary additive on haematology and water quality of common carp in a recirculating aquaculture system. In AIP conference proceedings. AIP Publishing LLC, 1888(1): 020023.

Guerreiro I., Serra C.L., Oliva-Teles A., Enes P. (2018). Short communication: gut microbiota of European sea bass (*Dicentrarchus labrax*) is modulated by short-chain fructooligosaccharides and xylooligo-saccharides. Aquaculture International, 26(1): 279-288.

Guerreiro I., Serra C.R., Ferreira P.P., Oliva-Teles A., Enes P. (2017). Prebiotics effect on growth performance, hepatic intermediary metabolism, gut microbiota and digestive enzymes of White Sea bream (*Diplodus sargus*). Aquaculture Nutrition, 24(1): 153-163.

Gupta A.; Gupta P., Dhawan A. (2014). Dietary supplementation of probiotics affects growth, immune responses and diseases resistance of *Cyprinus carpio* fry. Fish and Shellfish Immunology, 41(2): 113-119.

Gupta R., Jeevaratnam K., Fatima A. (2018). *Lactic Acid Bact*eria: Probiotic Characteristics, Selection Criteria, and Its Role in Human Health. Journal of Emerging Technologies and Innovative Research, 5(10): 411-424.

Harley J.P., Prescott L.M. (2002). Laboratory exercises in microbiology. 5th ed., The McGraw–Hill Companies, U.S.A. 449 p.

Hayek S.A., Gyawali R., Aljaloud S.O., Krastanov A., Ibrahim S.A. (2019). Cultivation media for lactic acid bacteria used in dairy products. Journal of Dairy Research, 86(4): 490-502.

Herwing N. (1979). Handbook of drugs and chemicals used in the treatment of fish diseases: A manual of fish pharmacology and materia medica. Thomas, Springfield. 272 p.

Iman M.K., Wafaa T., Abass T., Elham S., Awaad S., Mohammad M.N., Kawthc E., Gamal A., lbrahim A., Sade, Z., Elsayed H.S. (2013). Evaluation of *Lactobacillus plantarum* as a probiotic in aquaculture: Emphasis and growth performance and innate immunity. The Journal of Applied Sciences Research, 9(1): 572-582.

Imran S.M., Ail A.H., Najim S.M. (2019). Effect of Dietary Prebiotic Safmannan and Bio-antibiotic Fluconazole on some Growth and Haemato - immunological Parameters of Common Carp *Cyprinus carpio* L. Basrah Journal of Agricultural Sciences, 32(2): 176-192.

Ismael D.A.A.N. (2022). Isolation Probiotic Bacteria and Identification in Improving Immune Response of carp fish in Comparison with Commercial Product. Al-Kunooze Scientific Journal, 4(1):1.

Jibing M. (1993). Bioenergetics feed intake and energy partitioning. In: J.C. Rankin, F.B. Jensen, (Eds.), Fish physiology. London: Chapman and Hall. pp: 1-44.

Kafi Z.Z., Peyghan R., Modaresi S.M.H., Motevaseli E., Ghorbanpour M. (2022). Probiotic properties of some lactic acid bacteria isolated from intestine of cultured common carp, *Cyprinus carpio*, in Khuzestan Province, Iranian Veterinary Journal, 17(1): 86-96.

Kord M.I., Srour T.M., Omar E.A., Farag A.A., Nour A.A.M., Khalil H.S. (2021). The immunostimulatory effects of commercial feed additives on growth performance, non-specific immune response, antioxidants assay, and intestinal morphometry of Nile tilapia, *Oreochromis niloticus*. Frontiers in Physiology, 25(12): 627499.

Kumaree K.K., Akbar A., Anal A.K. (2015). Bioencapsulation and application of *Lactobacillus plantarum* isolated from catfish gut as an antimicrobial agent and additive in fish feed pellets. Annals of Microbiology, 65(3): 1439-1445.

Lambuk F., Mazlan N., Thung T.Y., New C.Y., Rinai K.R., Son R. (2022). A review of lactic acid bacteria isolated from marine animals: their species, isolation site and applications. Food Research, 6(1): 311-323

Lund R. (2021). Effects of functional ingredients from yeast in diets for Atlantic salmon (*Salmo salar*), from two genetic backgrounds, on growth performance and Nutrientutilization. Department of Animal and Aquacultural Sciences (IHA) Faculty of Biosciences. Master's Thesis. 49 p.

Magouz F.I., Ismail A., Radwan I.A., Soltan H.O., El-Keredy A. (2023). Synbiotic Lactic Dry® enhanced the growth performance, growth-related genes, intestinal health, and immunity of Nile tilapia reared in inland brackish groundwater. Annals of Animal Science, 1-29.

Majeed K.R., Ghadban A.K.G., Saleh F.M. (2017). A study of inhibition Activity of *Lactobacillus* spp. Against fungi and Aflatoxin B1 in vitro. Syrian Journal of Agricultural Research, 4(3): 65-79.

Maniat M., Salati A.P., Zanguee N., Mousavi S.M., Hoseinifar S.H. (2023). Effects of dietary *Pediococcus acidilactici* and Isomaltooligosaccharide on growth performance, immunity, and antioxidant defense in juvenile common carp. Aquaculture Nutrition, 1-8.

Meng X.L., Hu W.P., Wu S.K., Zhu Z.X., Lu R.H., Yang GK. (2019). Chinese yam peel enhances the immunity of the common carp (*Cyprinus carpio L.*) by improving the gut defence barrier and modulating the intestinal microflora. Fish and Shellfish Immunology, 95: 528-537.

Mohammed M.A., Al-Safao R.J.M. (2013). The effect of adding some probiotic bio-boosters in the attic to the performance of carp common in glass basins. Rafidain Agriculture Magazine, 41(2): 99-111.

Nieto-Domínguez M., de-Eugenio L.I., York-Durán M.J., Rodríguez-Colinas B., Plou F.J., Chenoll E., Pardo E., Codoñer F., Jesús-Martínez M. (2017). Prebiotic effect of xylooligosaccharides produced from Birchwood xylan by a novel fungal GH11 xylanase. Food Chemistry, 232: 105-113.

Nimalan N., Sørensen S.L., Feˇckaninova A., Koscova J., Mudronov D., Gancarcíkova S., Vatsos I.N., Bisa S., Kiron V., Sørensen M. (2023). Supplementation of lactic acid bacteria has positive effects on the mucosal health of Atlantic salmon (*Salmo salar*) fed soybean meal, Aquaculture Reports, 28: 101461

Noga E.J. (1996). Fish Disease: diagnosis and treatment. St Louis: Mosby-Year Book. 367 p.

Prückler M., Lorenz C., Endo A., Kraler M. Dürrschmid K., Hendriks K., Da Silva F.S., Auterith E., Kneifel W., Michlmayr H. (2015). Comparison of homo- and heterofermentative lactic acid bacteria for implementation of fermented wheat bran in bread. Food Microbiology, 49: 211-219.

Qaddoori M.S., Najim S.M., Al-Niaeem K.S. (2022). Effects of some probiotics and synbiotic dietary supplementation on growth performance and digestive enzymes activity of common carp, *Cyprinus Carpio,* Journal of Pharmaceutical Negative Results, 13(7): 175-184.

Qaddoori M.S., Al-Niaeem K.S., Najim S.M. (2023). Effects of Some Dietary Additives on Growth and Health Status of the Young Common Carp *Cyprinus carpio,* Egyptian Journal of Aquatic Biology and Fisheries, 27(2): 221-239.

Rane M., Markad A. (2015). Effects of Probiotics on the growth and survival Zebrafish *Danio rerio*. International Journal of Science and Research, 4(3): 1839-1841.

Rhema Z.A., Al-Noor J.M. (2022). Health and nutritional performance of young common carp *Cyprinus carpio* L. feeding diets with added bakery yeast Saccharomyces cerevisiae. International Journal of Health Sciences, 6(S6): 2424-2437.

Rialita T., Sukarminah E., Yuliana T., Sumanti D.M., Kurnianingrium I., Octaviani F.N., Santoso M.B., Susanto H.F. (2019). Isolation and Identification of Lactic Acid Bacteria Producing Bio preservative Bacteriosin from Smoked Fish, IOP Conf. Series: Earth and Environmental Science, 347: 1-5.

Ringø E., Hoseinifar S.H., Ghosh K., Doan H.V., Beck B.R., Song S.K. (2018). Lactic acid bacteria in finfish-An update. Frontiers in Microbiology, 9: 1-37.

Ringø E., Van Doan H., Lee S.H., Soltani M., Hoseinifar S.H., Harikrishnan R., Song S.K. (2020). Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. Journal of Applied Microbiology, 129: 116-136.

Rombenso A., Araujo B., Li E. (2022). Recent advances in fish nutrition: Insights on the nutritional implications of modern formulations. Animals, 12(13): 1705.

Salas-Leiva J., Opazo R., Remond C., Uribe E., Velez A., Romero J. (2017). Characterization of the intestinal microbiota of wild-caught and farmed fine flounder (*Paralichthys adspersus*). Latin American Journal of Aquatic Research, 45(2): 370-378.

Sevier H., Raae A.J., Lied E. (2000). Growth and protein turnover in Atlantic salmon (*Salmo salar*): the effect of dietary protein level and protein size. Aquaculture 185: 10-20.

Shao J., Wang B., Liu M., Jiang K., Wang L., Wang M. (2019). Replacement of fishmeal by fermented soybean meal could enhance the growth performance but not significantly influence the intestinal microbiota of white shrimp *Litopenaeus vannamei*. Aquaculture, 504: 354-360.

Singh G., Khati A., Chauhan R. (2021). Evaluation of vitamin C as growth for promoter's freshwater major, carp, *Cyprinus carpio*. Journal of Experimental Zoology Part A, 24(1): 377-382.

Soni M., Shah H.R., and Patel, S.M. (2019). Isolation, identification and analysis of probiotic characteristics of *Lactobacillus* spp. from Regional Yoghurts from Surendranagar District, Gujarat, Asian Journal of Dairy and Food Research, DR-1631: 1-6.

Suman G., Nupur M., Amerada S., Pradeep B. (2016). Single Cell Protein Production: A Review. Intern. International Journal of Current Microbiology and Applied Sciences, 4(9): 251-262.

Tacon A.G.J (1990). Standard methods for the nutrition and feeding of farmed fish and shrimp. In: Nutritive Sources and Composition. (2). Argent Laboratories Press, Redmond, W.A. 129 p.

Talbot C. (1985). Laboratory methods in fish feeding and nutritional studies. In: P. Tytler, P. Calow (Eds.). Fish energetics: New perspectives. Croom Helm, London and Sydney. pp: 125-154.

Taufik D., Arief M., Kenconojati H. (2019). The Effect of Different level of Probiotic Addition on Commercial Feed against Digestibility and Efficiency of Nile Tilapia Feed (*Oreochromis Niloticus*), IOP Conf. Series: Earth and Environmental Science, 236: 1-4.

Watts S.A., Lawrence A.L., Lawrence J.M. (2020). Nutrition. In: J.L. Lawrence (Ed.), Sea Urchins: Biology and Ecology, 4th. pp: 191-208.

Wuertz S., Schroeder A., Wanka K.M. (2021). Probiotics in fish nutrition — Long-standing household remedy or native nutraceuticals. Water, 13(10): 1348.

Xia Y., Cao J.M., Wang M., Lu M.X., Chen G., Gao F.Y. (2019). Effects of *Lactococcus lactis subsp. lactis* JCM5805 on colonization dynamics of gut microbiota and regulation of immunity in early ontogenetic stages of tilapia. Fish and Shellfish Immunology, 86: 53-63.

Xia Y., Wang M., Gao F., Lu M., Chen G. (2020). Effects of dietary probiotic supplementation on the growth, gut health and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*). Animal Nutrition, 6: 69-67.

Xiong J., Jin M., Yuan Y., Luo J.X., Lu Y., Zhou Q.C., Liang C., Tan Z.L. (2018). Dietary nucleotide-rich yeast supplementation improves growth, innate immunity and intestinal morphology of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Nutrition, 24: 1425-1435.