Hindawi
Aquaculture Nutrition
Volume 2023, Article ID 1451394, 16 pages
https://doi.org/10.1155/2023/1451394

Research Article

Effect of Beneficial Colonization of *Bacillus coagulans* NRS 609 on Growth Performance, Intestinal Health, Antioxidant Capacity, and Immune Response of Common Carp (*Cyprinus carpio* L.)

Jianxin Zhang, Mengyuan Huang, Junchang Feng, Yongyan Chen, Meng Li, Xiaolin Meng, and Xulu Chang

College of Fisheries, Henan Normal University, Xinxiang 453007, China

Correspondence should be addressed to Xulu Chang; changxulu19@163.com

Received 3 November 2022; Revised 24 May 2023; Accepted 29 May 2023; Published 14 June 2023

Academic Editor: Liqiao Chen

This study is aimed at exploring the effects of *Bacillus coagulans* NRS 609 on the growth performance, intestinal health, antioxidant activity, and immune response in common carp (*Cyprinus carpio* L.) by colonizing the intestinal tract with the beneficial strain. Gavage *B. coagulans* with GFP-tag at 10^9 CFU/mL into common carp for 7 days. Results indicated that *B. coagulans* were maintained in the intestine for at least 14 days. Four groups fed *B. coagulans* at different levels of inclusion, including 0 (CK), 1×10^7 (A), 1×10^8 (B), and 1×10^9 (C) CFU/g of feed for 8 weeks. Experimental groups showed significantly better growth performance than the control ($P < 0.05$). The treatment groups had higher digestive enzyme activity, villus height, and muscle thickness than the control ($P < 0.05$). Supplementation with *B. coagulans* also altered the composition of the intestinal flora. In addition, an enhancement of the antioxidant enzyme and a reduction of malondialdehyde in the liver were observed with the addition of *B. coagulans* ($P < 0.05$). Moreover, the cytokines of *IL-1β* and *IL-12*, *IL-10*, and *TGF-β*, significant upregulation in experimental groups ($P < 0.05$). Based on the results, the optimal level of *B. coagulans* was 10^7 CFU/g in diets for common carp.

1. Introduction

Common carp (*Cyprinus carpio* L.) has been farmed extensively in China for decades due to its nutritional qualities, delicious, and less expensive attributes [1, 2]. However, there is a risk of bacterial infection in fish raised in intensive systems [3, 4]. Several studies have demonstrated the effectiveness of antibiotics as feed additives in animal farming [5, 6]. Nevertheless, antibiotics are not recommended for disease control in aquaculture because of the environmental damage caused by antibiotic residues, bacterial resistance, the pollution of the environment, and negative consequences for the health of fish, resulting in food safety risks for human health [7–9]. Therefore, the search for alternative options to antibiotics is imperative for both the aquaculture industry and public health [10].

Probiotics are live microorganisms consisting of nonpathogenic microorganisms that benefit host animals by enhancing growth, immunity, and gut microbial balance [11–14]. Probiotics are being used as an alternative to antibiotics in aquaculture [13, 15, 16]. Several studies have documented the widespread use of probiotics in aquaculture, including lactic acid bacteria and yeast. Meanwhile, *Bacillus* spp. has been recognized as a developing trend [13, 17, 18].

In aquaculture, *Bacillus coagulans*, a spore-forming gram-positive bacterium, has frequently been applied as a feed additive due to its combination of lactic acid bacteria and *Bacillus* properties [19, 20]. Moreover, *B. coagulans* is known to endure high temperatures, acidity, and bile salts and inhibit enteropathogens [21–24]. Several studies have shown that *B. coagulans* improves growth, enhances immune function and microbiota regulation, and protects against disease [25, 26].

Previous studies have indicated that *B. coagulans* has beneficial effects on the organism by colonizing the intestine of animals, improving intestinal health, and resisting

pathogens [27]. Therefore, in the process of screening probiotics, the colonization ability under in vivo growth conditions has been paid more attention [28]. Probiotics can only exert their probiotic effects on the host when they have been colonized for a long period of time. The presence of additives facilitates the colonization of probiotics through an interaction with the intestinal flora. Therefore, the adhesion ability and colonization of probiotics have become important criteria for screening probiotics [29].

A green fluorescent protein (GFP) label can be used to allow bacteria to be visible within the host gut and to trace their activities, including adhesion and colonization [30, 31]. GFP, a small protein weighing 27 K Da, is capable of fluorescing when exposed to ultraviolet (UV) light. Under UV light, bacteria modified with the exogenous GFP gene are readily recognizable. The GFP protein's fluorescence does not depend on cofactors, substrates, or any other gene products, making it sensitive, stable, specific, nontoxic, and noninterfering with cell growth and function. [32, 33]. Previous research has established the utility of GFP and used GFP-marked probiotics to study interaction in vivo and in vitro fish models [34].

Most importantly, this study primarily focused on investigating the colonization capacity of *B. coagulans* NRS 609 in the intestine of common carp and investigating the effects of dietary *B. coagulans* NRS 609 on the growth performance, antioxidant capacity, intestinal microflora and health (morphology and digestive enzyme activity), and disease resistance against *Aeromonas hydrophila* of common carp.

2. Materials and Methods

2.1. Diet Preparation. The *B. coagulans* NRS 609 strain was obtained from the China Center for Type Culture Collection (CCTCC) located in Wuhan, Hubei Province, China. The bacteria were streaked and revived in MRS broth under sterile conditions. A 1 : 1 ratio of bacterial solution to glycerol was used, with 60% glycerol used, stored at -80°C in the microbiological preservation laboratory of the College of Fisheries at Henan Normal University. Morphological and biochemical characteristics of the bacterium were used, as well as an analysis of the 16S rRNA gene, to identify it. The strain was cultured at 42°C in MRS broth for the preparation of competent cells. The plasmid pAD123-pgal-GFP was gifted from the College of Life Sciences, Henan University, a variant of GFP with a red-shifted excitation peak at 488 nm and maximal emission at 507 nm. *B. coagulans* NRS 609 with GFP-tag was constructed by introducing the plasmid pAD123-pgal-GFP, named recombinant *B. coagulans* [35]. The recombinant *B. coagulans* were incubated at 42°C with shaking for 48 h in MRS broth. Approximately centrifuged at 8000 × g for 10 minutes, washed three times with pH 7.4 phosphate buffer saline, and then resuspended in sterile water to achieve the desired concentration of 1×10^9 CFU/mL. The *B. coagulans* colonization ability was determined by gavaging the prepared bacterial suspension in the intestine of common carp.

The feeding experiment was set up in four groups for the determination of *B. coagulans* effects in common carp. During 48 h of incubation at 42°C, *B. coagulans* strains with shaking in MRS broth. Next, the cultures were subjected to centrifugation for 10 min at a speed of 5000 × g. To resuspend the bacteria, three times sterile phosphate buffer saline was adjusted to 1×10^9 CFU/mL. The bacterial suspension was diluted and evenly sprayed at different concentration gradients (0 (control diet, PBS), 10^7, 10^8, and 10^9 CFU/g) on the basal diet (Table 1), named CK, A, B, and C. The diets were subsequently dehydrated and kept at -20°C for feeding. And the actual count of bacteria in the formulated diets was sampled to test by adopting the method of gradient dilution coating whether it met the requirements of this experiment. Exactly, 1 g formulated diet was mixed with 9 ml sterile PBS, then serially diluted. After that, a 0.1 mL aliquot was spread on MRS broth agar plates and incubated for 48 h at 42°C. Plates with colony numbers between 30 and 300 were used to calculate and confirm the actual count of the bacteria in the formulated diets, which was recorded in stages and monitored effectively to ensure the meticulousness of the process and the accuracy of the results.

2.2. Experimental Conditions. The common carp was acquired from a Freshwater Aquaculture Institute located in Henan Province in China. The basal diet was given to healthy fish that had been acclimatized in 300 L tanks under controlled lab conditions at 26-27°C for two weeks.

Since then, routine physical and microbiological examinations have been conducted on these fish which are normally fed, disease-free and uninjured to guarantee that there are no bacterial diseases or any abnormal clinical symptoms. For the gavage trial, thirty healthy common carp of similar size (62.36 ± 3.80 g) were selected and placed into two tanks with fifteen fish in each 100-L tank. Next, the experimental group received 0.5 mL of recombinant B by gavage. Administer *B. coagulans* suspension (10^9 CFU/mL) once daily for a duration of 7 days. In the control group, the same volume of *B. coagulans* suspension was lacking plasmid DNA. A basal diet was provided to the fish three times a day (08 : 00, 12 : 00, and 16 : 00).

Furthermore, 240 healthy common carp, with similar sizes (32.50 ± 1.52 g), were randomly divided into twelve 100 L tanks, with 20 fish in each tank. This was replicated in three tanks per treatment for the feeding trial. After the trial began, each group of fish was fed its respective diet three times daily (at 08 : 00, 12 : 00, and 16 : 00). For 8 weeks, 3% of the common carp body weight was fed daily. After the second week, the feed rate was adjusted based on body weight.

During the trial, around 30% of the cultured water was replaced once a day with dechlorinated, fresh water at the same temperature, with oxygen saturation maintained by aeration. Furthermore, the fish were kept in tanks of 100 L size that received constant water flow, while being subjected to natural photoperiod conditions. The water temperature ranged between 25.0 and 29.0°C, pH levels were maintained between 7.2 and 7.6, the dissolved oxygen level was maintained above 5 mg/L, and the nitrogen concentration was restricted to less than 0.05 mg/L. A collection of uneaten diets was made, dried, and weighed.

Table 1: Ingredients and nutritional composition of the basal diet (% dry matter).

Ingredients	Composition
Fish meal	20.00
Flour	20.00
Soybean meal	20.00
Casein	19.00
CMC-Na	10.00
Cottonseed meal	5.00
Soybean oil	3.00
CaH_2PO_3	1.50
Mineral mixture	1.00
Methylcellulose	0.20
Salt	0.20
Vitamin mixture	0.10

Note: purchase from Tongwei Group Co., Ltd., Xinxiang, Henan Province, China.

2.3. The Growth Dynamics of B. coagulans in Common Carp. After 7 days of gavage, resume normal daily feeding. Three fish and their intestines were, respectively, taken from the experimental group at 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, and 14 d days after stopping gavage for imaging analysis by an animal in vivo imaging system (Aniview100, Yunxing Scientific Instruments Co., Ltd, Guangzhou Province, China). Furthermore, we took three fish and their intestines from the control group. The 200 ppm MS-222 solution was used to euthanize these fish (purchased from Aladdin Bio-chemical Technology Co., Ltd, Shanghai, China). Then, the interior of the foregut, midgut, and hindgut were washed with PBS and, respectively, collected in centrifuge tubes for imaging analysis and colony count. The intestine samples were ground and spread on LB medium with ampicillin for 24 h, and the number of *B. coagulans* labelled with green fluorescence was observed through a fluorescence microscope (LIFA, Aunion Tech Co., Ltd, Shanghai, China).

2.4. Growth Performance. The following formulas were used to calculate the initial body weight (IBW), final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), and feed conversion ratio (FCR):

$$\begin{aligned} \mathrm{WGR} &= 100\% \times \frac{\mathrm{FBW} - \mathrm{IBW}}{\mathrm{IBW}}, \\ \mathrm{SGR} &= 100\% \times \frac{\ln\mathrm{FBW} - \ln\mathrm{IBW}}{\mathrm{days}}, \\ \mathrm{FCR} &= \frac{\text{feed intake(g)}}{\mathrm{FBW} - \mathrm{IBW}}. \end{aligned} \tag{1}$$

2.5. Sample Collection. A growing trial of 8 weeks was followed by a 24-hour fast before samples were collected. Eighteen fish per dietary treatment (six fish per tank) were selected at random and euthanized with MS-222 solution at 200 ppm. Then, the liver and intestinal tissue of fish were dissected under aseptic conditions with surgical scissors. Moreover, aseptic scraping, snap-freezing, and freezing intestinal contents were performed. After washing the intestine with sterile PBS (pH 7.4), the tissue samples were kept at -80°C for further analysis.

2.5.1. Analysis of Digestive Enzyme Activities. Homogenized foregut, midgut, and hindgut samples from common carp were homogenized with 0.9% aseptic saline in a ratio of 1 : 9 (tissue: saline) on ice for 10 min. To analyze the digestive enzyme activity, the homogenate was centrifuged for 10 minutes at 4°C at 3,000 rpm and collected in 1.5 mL centrifugal tubes.

To determine protease, amylase, and lipase activities, commercial kits were obtained from the Nanjing Jiancheng Bioengineering Institute in China. Protease activity was determined based on its ability to enter the chain of hydrolysis substrate ethyl arginine at 253 nm [36]. Amylase was estimated based on the hydrolysis capacity of the unhydrolysed starch with iodine solution to produce relatively stable brownish-red compounds and measured at a 660 nm absorption peak. To quantify the level of amylase activity, a solution was used to reveal nonhydrolyzed starch [37]. The lipase activity was assessed by assessing its hydrolysis capability of triglyceride and its ability to produce a cloudy emulsion when combined with water, and then determining the absorption peak at 420 nm [38].

2.5.2. Morphological Analysis. A 4% paraformaldehyde solution was used to fix the intestinal tissues. They were routinely processed, sectioned transversely at 6 μm, and then stained with hematoxylin and eosin (H&E) under a light microscope (Nikon Eclipse E400). The tissue slides were utilized to measure the villus height and muscular thickness of the intestine wall. 9 intestinal samples of each group were used to measure both villus height and muscular thickness. Each intestinal sample had 3 measurements of villus height and muscular thickness.

2.5.3. Intestinal Microbiota Analysis. The QIAamp PowerFecal DNA Kit was used to extract intestinal microbe DNA, in accordance with manufacturer instructions. Then, the DNA was amplified from the V3-V4 hypervariable region of the 16S rRNA using universal primers 338F (ACTCCTACGGG AGGCAGCA) and 806R (GGACTACHVGGGTWTCTA AT). Amplification of the 16S rRNA gene sequence by PCR, the PCR program was conducted according to the volume and reaction program previously reported by Chang et al. [39]. The sequencing was performed on an Illumina MiseqTM PE 300 system with high throughput (Shanghai Majorbio Bio-pharm Technology Co., Ltd).

In order to acquire clean, high-quality reads, after quality-filtered the raw reads. Additionally, for OTUs, a sequence difference threshold of 97% was established. Community composition was shown by PCoA (principal coordinate analysis), whereas the significant differences among species were shown by the Kruskal-Wallis H test. The bar diagram was used for assessing the microbe the samples contain at a certain taxonomic level and the relative abundance of the microbes in the samples.

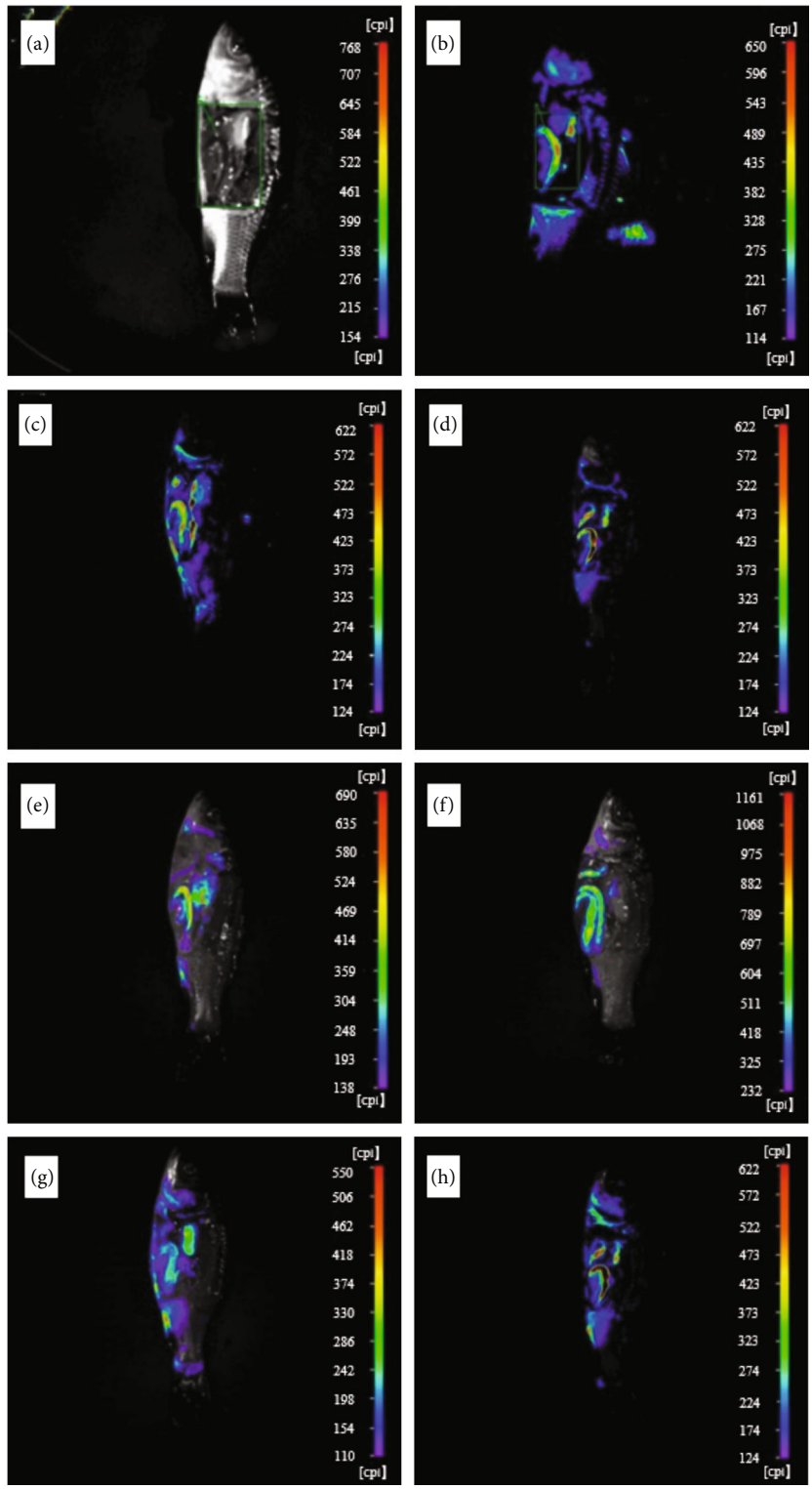

Figure 1: The growth dynamic of *Bacillus coagulans* in common carp. (a) Fish body imaging picture after gavage in the control group; (b–h) Fish body imaging pictures of the experimental group at 2, 4, 6, 8, 10, 12, and 14 days after gavage.

2.5.4. Liver Antioxidant Analysis. A homogeneous sample of 1 g liver tissue was mixed with 9 g of PBS (0.01 M, pH 7.4) on ice. The supernatant was centrifuged for 10 minutes at 4°C to separate it carefully for subsequent analysis.

A commercial kit (provided by Nanjing Jiancheng Biological Engineering Institute, China) was used to evaluate the total antioxidant capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA)

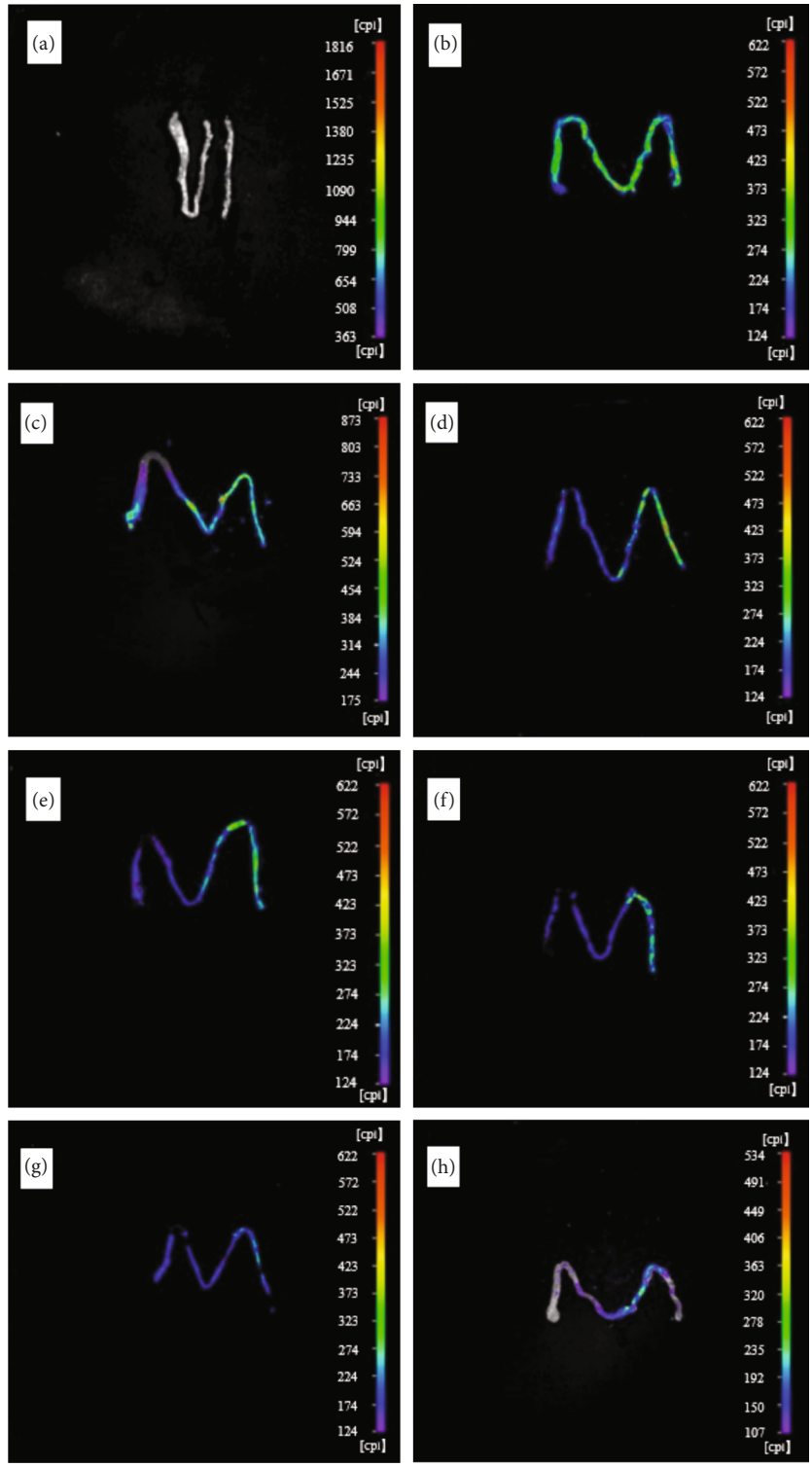

FIGURE 2: The growth dynamic of *Bacillus coagulans* in the intestine of common carp. (a) Intestinal imaging photograph of the control group. (b–h) Intestinal imaging photos of the experimental at group 2, 4, 6, 8, 10, 12, and 14 days after gavage.

on a Microplate Reader (PerkinElmer, USA). The T-AOC activity was assessed by measuring its capacity to reduce Fe^{3+} to Fe^{2+} with antioxidant substances, leading to complex formation with phenanthrolines. This complex was quantified at a 520 nm absorption peak. To perform the catalase (CAT) assay, hydrogen peroxide (H_2O_2) was spectrophotometrically determined with ammonium molybdate that absorbs at 405 nm. In order to determine the SOD activity, nitrite and superoxide anion radicals were inhibited by SOD. In this process, purplish red compounds were produced at a wavelength of 550 nanometers. The TBA method was employed for the detection of MDA in a glacial acetic

acid solution. Condensing the decomposition products of lipid hydroperoxide with TBA can yield red compounds with an absorption peak at 532 nm.

2.6. A. hydrophila Challenge Experiment. *A. hydrophila* was provided by the Henan Normal University's aquatic animal disease control center from infected common carp. A culture of *A. hydrophila* was grown in a 250 mL flat bottom flask using 100 μL of Luria broth. After 12 h of incubation at 37°C and 180 rpm, the cells were centrifuged (7,000 rpm for 10 minutes at 4°C) and pelleted. The supernatant was eliminated and PBS was used to reintroduce the bacterial cells, which were then diluted to achieve varying concentrations of *A. hydrophila* during the 7-day experiment. Upon completion of the 8-week feeding trial, another three fish from each replicate were randomly selected and injected intramuscularly with 100 μL of suspended bacteria ($LD_{50} = 1 \times 10^8$ CFU/mL) at the third abdominal segment for the disease resistance test. Nine common carps were injected with PBS as a negative control (K-). As a positive control, 100 μL of *A. hydrophila* ($LD_{50} = 1 \times 10^8$ CFU/mL) were injected. In accordance with the conditions described earlier, 24 h after infection with *A. hydrophila*, the caudal vein was punctured for the collection of blood. After centrifuging at 3,500 rpm/min at 4°C for 15 min. A microplate reader (Perkin Elmer, USA) was used to perform ELISA with cytokine polyclonal antibodies. To summarize, the procedure involved coating a Nunc 96-well Immuno-Maxisorb plate was similar to those of Feng et al. [40].

2.7. Statistical Analyses. The results were demonstrated in the form of the mean plus the standard deviation (SD) of three repeated measurements. An analysis of one-way variance was conducted for analysis purposes, followed by a multiple-range test using Duncan's formula. The statistical significance was determined with a threshold of $P < 0.05$. The tank means ($n = 6$ per treatment) were used as statistical units.

3. Results

3.1. The Growth Dynamics of B. Coagulans in Common Carp. Figures 1 and 2 show the results. As can be seen in Figure 1, except for the control group. All fish displayed fluorescence signals in other pictures. Combined with Figure 2, the observation of stripping the intestinal tract revealed that the fluorescence signals inside the intestines remained intact for 2-14 days. Fluorescence signals weaken over time. In Figure 3, there is a gradual downward trend in fluorescence intensity in the fish and the intestine. After gavage, the midgut fluorescence signal was stronger than other parts of the intestines. However, the slowly decreasing hindgut fluorescence intensity remained relatively stable.

Table 2 shows the total number of recombinant *B. coagulans* in common carp intestines. The bacteria count remained high for the entire 6-day period. However, on the 8th day, the *B. coagulans* count sharply dropped. There was always a detectable bacterial count in all three parts of the intestine during the 14-day period. On the 14th day, the bacterial count in the foregut diminished dramatically to only 2.00×10^2 CFU/g due to prolonged time. By the 4th day, the midgut bacteria had decreased, and by the 8th, they had stabilized. The overall number of bacteria in the hindgut exhibited a decreasing trend, albeit modest. There were still 2.00×10^3 CFU/g of recombinant *B. coagulans* on the 14th day. This was more stable than in the foregut and midgut.

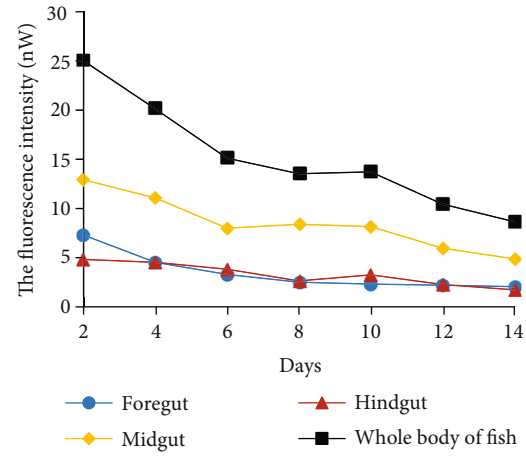

Figure 3: Variation trend of *Bacillus coagulans* in various parts of common carp.

Table 2: The count of *Bacillus coagulans* in the intestine.

Days after gavage	Foregut	Midgut	Hindgut
	Total bacteria count (CFU/g)		
2	3.34×10^6	6.10×10^6	3.47×10^4
4	2.06×10^4	8.33×10^4	5.83×10^4
6	2.67×10^4	5.50×10^4	3.50×10^4
8	2.70×10^3	3.10×10^3	5.93×10^3
10	2.20×10^3	2.30×10^3	3.14×10^3
12	1.40×10^3	2.60×10^3	2.87×10^3
14	2.00×10^2	1.10×10^3	2.00×10^3

3.2. Growth Performance. Table 3 presents growth performance parameters. Upon feeding fish diets containing *B. coagulans*, all harvest growth parameters (i.e., FBW, WGR, and SGR) exceeded those of the control group by a significant margin ($P < 0.05$). Contrarily, the FCR of fish given diets containing *B. coagulans* groups was significantly lower than that of the control group ($P < 0.05$). The fish belonging to group A demonstrated superior growth indices, with a weight gain rate of 257.10, specific growth rate of 2.12, and feed conversion rate of 1.03 ($P < 0.05$).

3.3. Digestive Enzyme Activities. Figure 4 illustrates the impact of dietary supplementation with *B. coagulans* on digestive enzyme activity in the intestine. Comparing group C with the control group, protease activity in the intestines improved dramatically ($P < 0.05$). As for the amylase activity

Table 3: Effects of *Bacillus coagulans* on growth performance of common carp.

Growth parameter	Concentration of *Bacillus coagulans*			
	CK	A	B	C
IBW (g)	33.30 ± 0.52	32.16 ± 0.38	32.66 ± 0.25	31.87 ± 0.50
FBW (g)	90.16 ± 7.90^{c}	114.84 ± 17.96^{a}	103.31 ± 6.36^{b}	101.14 ± 14.17^{b}
WGR (%)	171.10 ± 9.05^{c}	257.10 ± 2.29^{a}	216.37 ± 4.69^{b}	217.53 ± 7.40^{b}
SGR (%)	1.65 ± 0.04^{c}	2.12 ± 0.01^{a}	1.92 ± 0.03^{b}	1.92 ± 0.04^{b}
FCR	1.36 ± 0.05^{a}	1.03 ± 0.02^{c}	1.20 ± 0.04^{b}	1.17 ± 0.02^{b}
Survive (%)	100.00	100.00	100.00	100.00

Note: control (CK): 0 CFU/g; group A: 10^{7} CFU/g; group B: 10^{8} CFU/g; group C: 10^{9} CFU/g. Values with same superscript letters in the same column are not significantly different, while different letters are significantly different ($P < 0.05$).

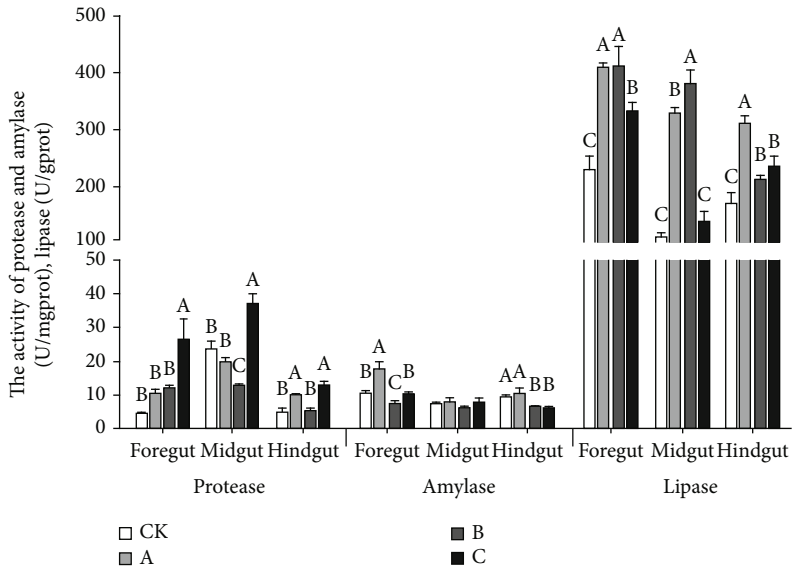

Figure 4: The digestive enzyme activities in common carp. Adding *B. coagulans* to 1 g commercial diet: control (CK), 0 CFU/g; group A, 10^{7} CFU/g; group B, 10^{8} CFU/g; and group C, 10^{9} CFU/g. The values marked with different letters are significantly different ($P < 0.05$).

in the intestines, both the foregut and hindgut of group A increased significantly compared to the control group ($P < 0.05$), but not among the midgut of all groups ($P > 0.05$). Supplementation of a diet with *B. coagulans* had increased growth in both their foregut and hindgut. There was a significant difference between the experimental and control groups in lipase activity ($P < 0.05$). Groups A and B showed higher lipase activity (significantly higher than control), but groups C and the control showed no significant difference ($P > 0.05$).

3.4. Intestinal Morphology. The histological status of the midgut is shown in Figure 5. In the control group and groups B and C (images (d), (b), and (c)), there were revealed twists and fusions of intestinal villi. However, group A showed a tendency towards a regular arrangement of intestinal villi, with little or no observed twisting and fusion (image (a)).

Figure 6 presents intestinal morphometric parameters. A significant increase in villi height was observed in the midgut and hindgut of fish supplemented with *B. coagulans* in comparison to those fed a control diet ($P < 0.05$). As compared to the other groups, group B had significantly higher villi height in the foregut. Additionally, all treatment groups displayed significantly increased muscle thickness compared to the untreated group ($P < 0.05$).

3.5. Intestinal Microbiota Analysis. A total of 549,704 raw reads were produced by high-throughput sequencing. With quality filtering and merging, we obtained 528,841 trimmed sequences from 12 samples, on average with a length of 428 bp. Figure 7 shows that the OTU rarefaction curve, based on a similarity cut-off of 97%, was leveled off, indicating sufficient sequencing depth.

A PCoA analysis was conducted using the pyrosequencing data to assess β-diversity. As shown in Figure 8, the groups treated with *B. coagulans* exhibited structural dissimilarity from CK. The primary principal component (PC) scores are PC1 = 49.04% and PC2 = 24.13%. There was an evident division of the microbial community in the intestine into four groups, and each group was gathered, indicating that the microorganisms in each group had certain similarities and the community structures of the different groups differed in certain respects.

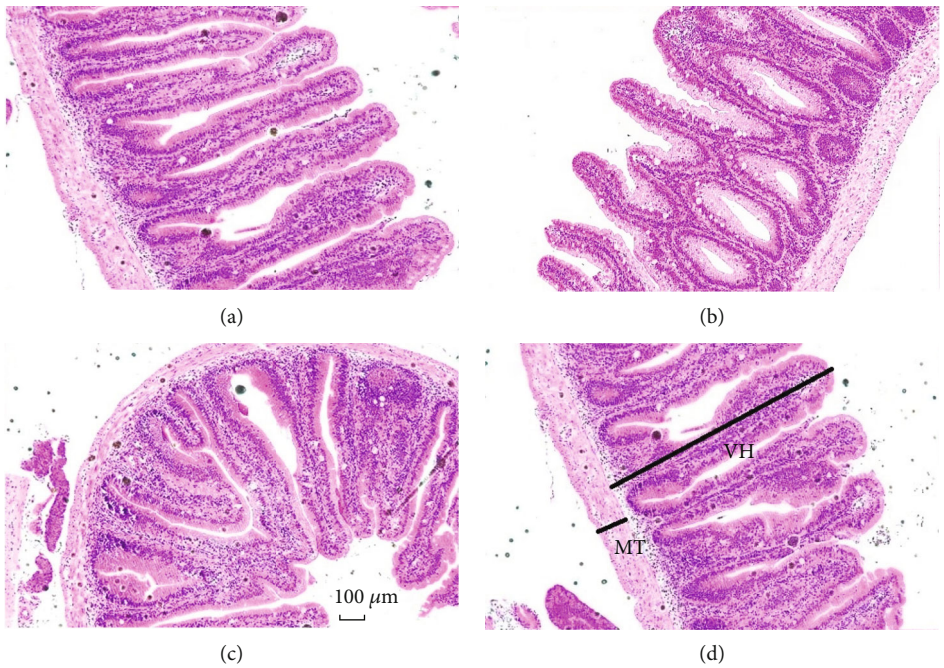

Figure 5: Photomicrographs and histological measurements of the midgut cross-cutting of common carp fed with different concentrations of *B. coagulans*: (a) 10^7 CFU/g; (b) 10^8 CFU/g; (c) 10^9 CFU/g; (d) control. VH = villus height; MT = muscle thickness. Scale bar = 100 μm.

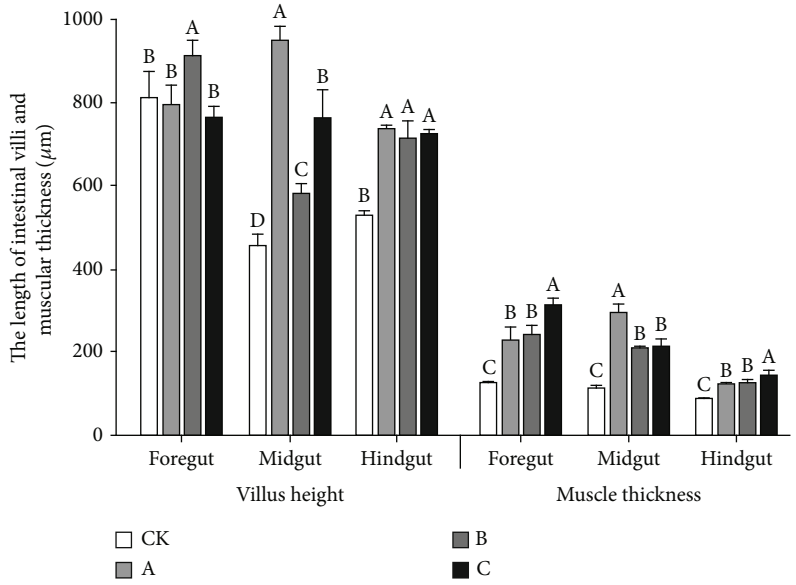

Figure 6: The length of intestinal villi and muscular thickness in common carp. Adding *B. coagulans* to 1 g commercial diet: control (CK), 0 CFU/g; group A, 10^7 CFU/g; group B, 10^8 CFU/g; and group C, 10^9 CFU/g. The values marked with different letters are significantly different ($P < 0.05$).

Through phylogenetic analysis of the sequence, all sequences belonged to 18 phyla based on the phylum level. The 12 intestinal samples included CK1, CK2, CK3, A1, A2, A3, B1, B2, B3, C1, C2, and C3, with *Proteobacteria* (97.85%), *Firmicutes* (1.49%), and *Bacteroidetes* (0.31%) being the main organisms present. The total sequence was accounted for by these sequences at a rate of 99.65%. These groups were the main dominant flora of intestinal microbes. The specific distribution is shown in Figure 9. The addition of *B. coagulans* significantly increased Firmicutes among them, reaching the highest value in Group C, while *Bacteroides* decreased with increasing concentrations of *B. coagulans* and disappeared in group C.

At the genus level, all sequences belong to 151 genera, and the species distribution of each group is shown in Figure 10. The dominant species of the intestinal flora was

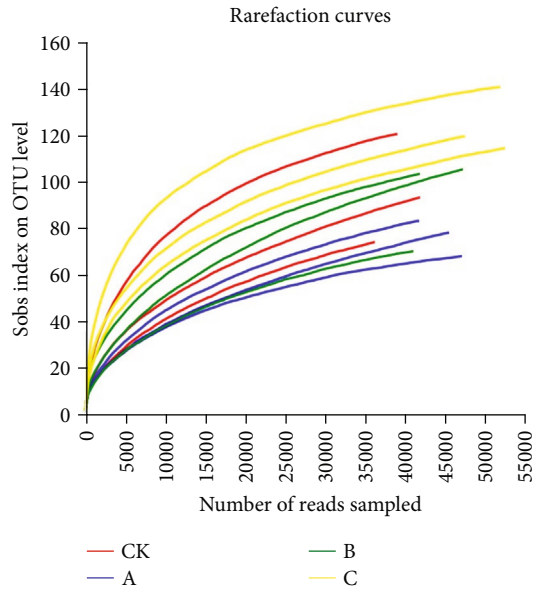

Figure 7: Rarefaction curves. Adding *Bacillus coagulans* to 1 g commercial diet: control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; and group C: 10^9 CFU/g.

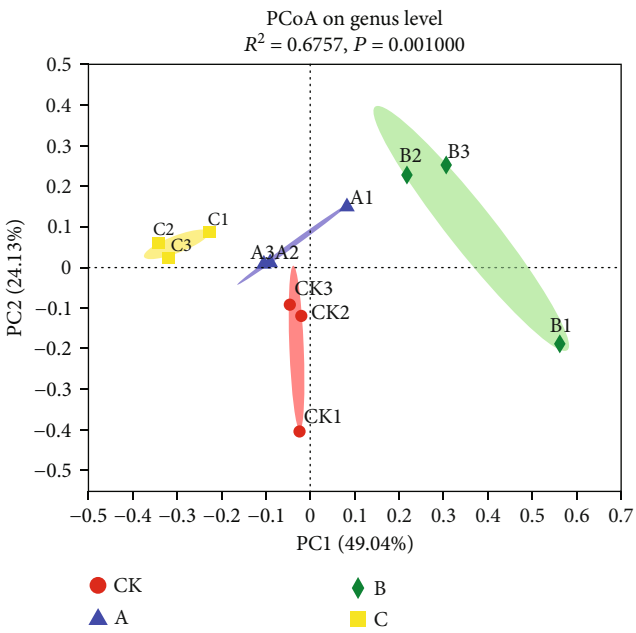

Figure 8: Control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; group C: 10^9 CFU/g. Principal coordinates analysis of the Spearman-approx scores of the microbial communities. Principal components (PCs) 1 and 2 explain 49.04% and 24.13% of the variance, respectively.

Aeromonas (81.54%). *Shewanella* proportions decreased with increasing concentrations of *Bacillus coagulans*, while *Gemmobacter* and *Bacillus* proportions increased.

After supplementing the diet of common carp with *B. coagulans* for eight weeks, the researchers used Kruskal-Wallis tests to examine the changes in gut microbiota at both the phylum and genus levels. At the phylum level (Figure 11), they demonstrated that there were no notable differences in the prevalence of *Proteobacteria* ($P = 0.075$) and *Bacteroidetes* ($P = 0.034$), *Chloroflexi* ($P = 0.183$) among the four groups ($P > 0.05$). With rising concentrations of *B. coagulans*, the abundance of *Firmicutes* showed a gradual increase that was significant ($P = 0.050$). *Actinobacteria* ($P = 0.048$) increased significantly in the experimental groups, and *Chlamydia* ($P = 0.050$) increased significantly in group C. At the genus level, in the experimental groups, the relative abundances of *Gemmobacter* ($P = 0.016$), *Bacillus* ($P = 0.022$), *Bosea* ($P = 0.038$), norank_f__*Rhizobiales*_*Incertae*_*Sedis* ($P = 0.033$), unclassified_f__*Rhodobacteraceae* ($P = 0.019$), *Rhodobacter* ($P = 0.019$), and norank_f__*Desulfovibrionaceae* ($P = 0.025$) were increased significantly when compared to the control group ($P < 0.05$), as indicated by the value of Figure 12.

3.6. Liver Antioxidant Analysis. Figure 13 shows a liver oxidative indicator in common carp. group C displayed significantly higher T-AOC activity than other groups ($P < 0.05$). The improvement in CAT was significantly greater in groups B and C compared to the control group, while it was relatively lower in group A. SOD levels in the liver were significantly higher in groups A and B than in control and group C ($P < 0.05$). Conversely, the oxidative damage indicator MDA decreased dramatically across all *B. coagulans*-supplemented groups ($P < 0.05$), without significant differences between the experimental groups ($P > 0.05$).

3.7. A. hydrophila Challenge Experiment. After the 7-day challenge with *A. hydrophila*, the results are shown in Figure 14. The treated groups secreted significantly more proinflammatory factors *IL-1β* and *IL-12* than the control group ($P < 0.05$), with group A demonstrating the strongest activity. And the secretion of *IL-1β* and *IL-12* was remarkably upregulated in the positive control group than in the K-group. For anti-inflammatory cytokines, the experimental groups showed significantly higher expression levels of *IL-10* and *TGF-β* ($P < 0.05$). The positive control group exhibited a significant decrease in *IL-10* and *TGF-β* expression compared to the negative control group ($P < 0.05$).

4. Discussion

B. coagulans is a promising probiotic candidate among the many probiotics used as a protein feed additive in the aquaculture industry because of its nonpathogenic and nontoxic properties [16, 41]. Currently, there is a growing field of research focusing on the colonization ability of probiotics, which can adhere to and colonize the tissue surface of the organism, leading to various prebiotic functions [34]. But there is no report that has been directed towards the colonization ability of *B. coagulans* to common carp.

Previous studies have shown that probiotics have different colonization ability in the intestines, generally between 2 and 65 days [42, 43]. According to this study, the results showed that *B. coagulans* established colonization in the common carp intestine within 2-14 days. Xu et al. [44] study generally agreed with this, who found that the colonization

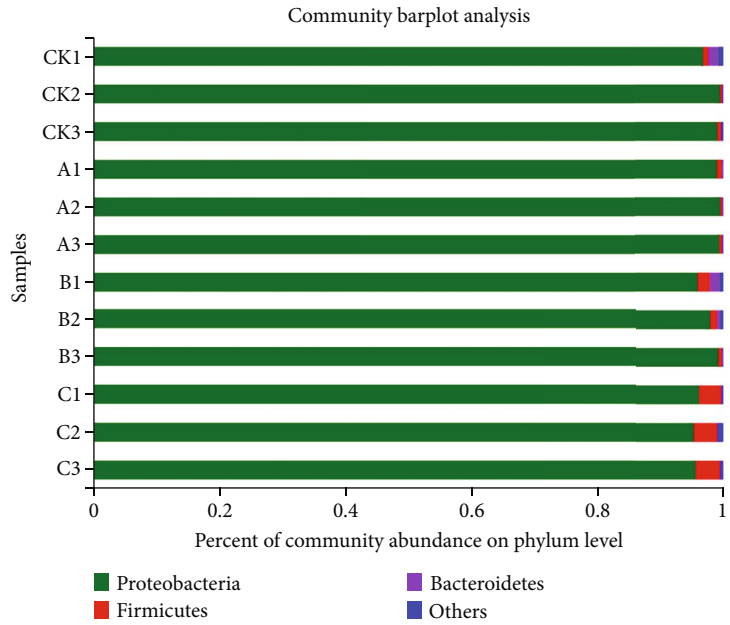

Figure 9: Bacterial composition of the different communities at the phylum level. Adding *Bacillus coagulans* to 1 g commercial diet: control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; and group C: 10^9 CFU/g.

of *Bacillus subtilis* could survive and colonize the intestine of zebrafish, but the colonization ability was not explained. In our study, the trend of survival dynamics in *B. coagulans* was similar to the observation of Ringo and Gatesoupe [45]. This may be due to the metabolism of recombinant *B. coagulans* present in the intestine suggests a colonization rate of *B. coagulans* in the midgut was relatively high. To determine whether *B. coagulans* has an effective function on common carp, further study about the probiotic effect after the colonization of *B. coagulans* in the intestine.

The current study assessed the impact of *Bacillus coagulans* supplementation on the growth performance, intestinal health, antioxidant capacity, and immune response of common carp through beneficial colonization in the intestine. There has been evidence that supplementing animal diets with *Bacillus* improves growth performance [19, 46, 47]. Furthermore, Sadeghi et al. [48] found that feeding fish with a combination of *B. licheniformis* and 3% lemon peel resulted in the most effective growth performance. Amoah et al. [19] improved the WGR and SGR of *Litopenaeus vannamei* with dietary supplementation with *B. coagulans*. Furthermore, our findings align with multiple studies conducted on other animals such as broiler chickens and piglets. These studies have demonstrated that diets enriched with *Bacillus* spp. enhance growth performance [49–51]. It is hypothesized that the common carp's enhanced growth performance is due to the release of digestive enzymes by *B. coagulans,* which improve feed digestion [16].

Fish digestion and absorption depend heavily on the intestines [52]. Several morphological characteristics of the gut (villi height, muscular) and digestive enzymes (protease, amylase, and lipase) indicate the presence of a healthy gut within fish [53]. It is also believed that the higher or wider the microvilli of the intestinal epithelial cells, the broader the surface area for absorbing nutrients [54, 55]. The current research demonstrated a noteworthy rise in both the villi height and muscular thickness of probiotic groups in comparison to the untreated group, which is corroborated by Amoah et al. [19] findings. In previous studies, *Bacillus* was added to the diets of several fish species, including triangular bream (*Megalobrama terminalis*) as described by Zhang et al. [56] and Nile tilapia (*Oreochromis niloticus*) as reported by Han et al. [57]. Coincidentally, in our study, improvements in protease, amylase, and lipase activities were detected in the diet-supplemented *B. coagulans* group were higher than the control in various degrees, but not all improvements were significant. Similar results were obtained in *Litopenaeus vannamei* with improved digestion enzyme activity [58]. The improvement of common carp with dietary supplementation *B. coagulans* on villi length and muscular thickness may be associated with digestive enzyme activity, which improves feed digestibility. Higher rates of growth can be attributed to higher digestive enzyme activity, which results in increased absorption capacity through the breakdown and nutrient uptake by digestive enzymes [59].

The intestinal microflora was crucial to shape the structure [60, 61]. As a result, they may contribute to the improvement of the inflammatory response by forming a protective barrier that prevents pathogens from entering [60, 62, 63]. The results of our study indicated that there was a profound impact of *B. coagulans* on the composition and diversity of the intestinal microbiota of common carp, as demonstrated by the number of OTUs analyzed, as well as PCoA analysis and an increase in the diversity of alpha. By adding *B. coagulans* significantly elevated the abundance of *Firmicutes* and lowered the ratio of *Bacteroides.* The results of Amoah et al. [19] parallel this. Several species of the genera *Aeromonas*, *Shewanella*, *Gemmobacter*, and *Bacillus* were dominant at the genus level in this study.

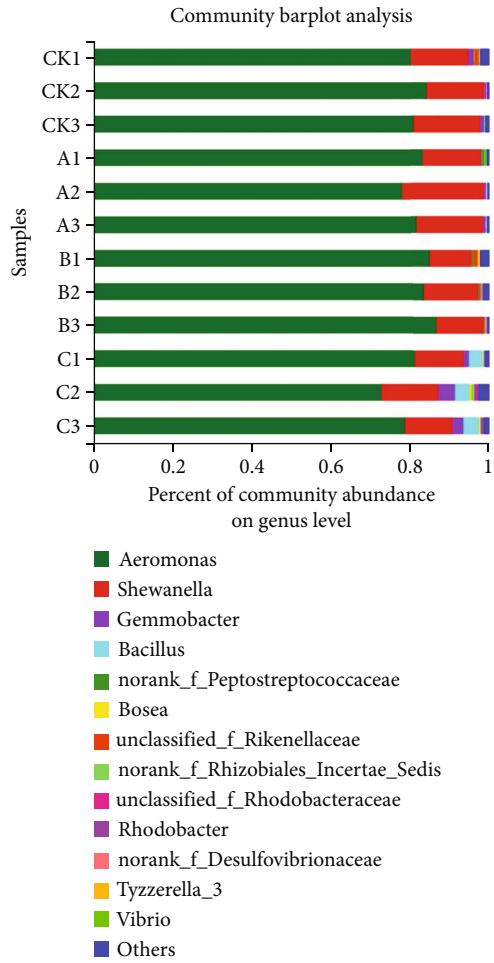

Figure 10: Bacterial composition of the different communities at the genus level. Adding *Bacillus coagulans* to 1 g commercial diet: control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; and group C: 10^9 CFU/g.

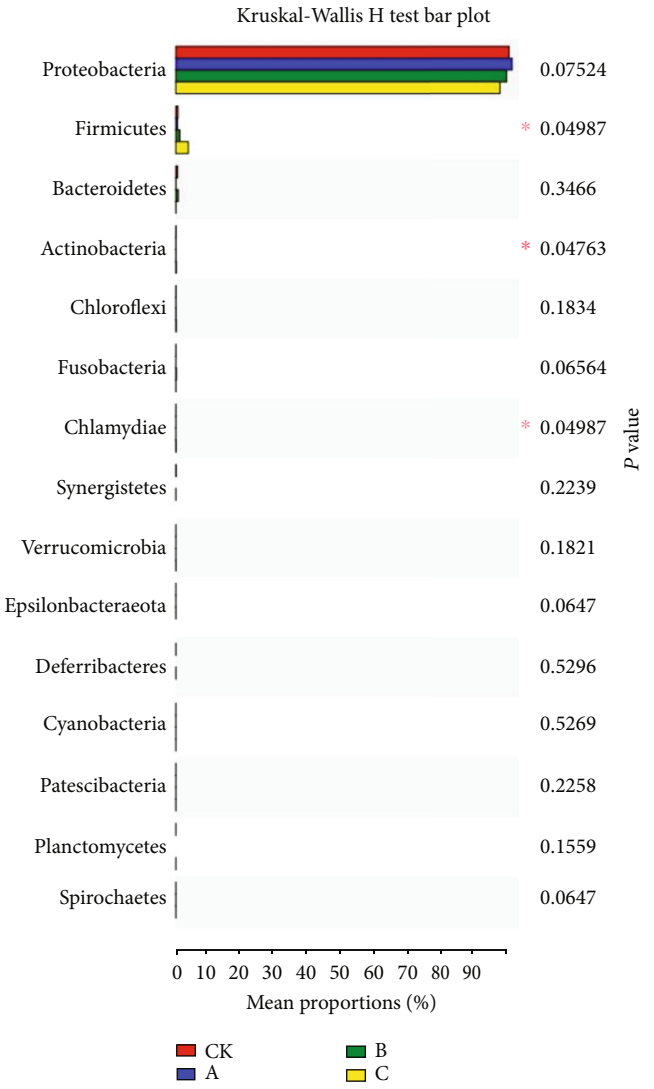

Figure 11: Species diversity analysis at phylum level. Adding *Bacillus coagulans* to 1 g commercial diet: control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; and group C: 10^9 CFU/g.

Shewanella is a gram-negative bacterium that mainly infects seafood such as fish and shrimp and makes them corrupt and fester [64, 65]. The study showed that common carp fed a *balanced diet supplemented with B. coagulans* had significantly lower Shewanella levels. Meanwhile, the proportion of *Bacillus* increased gradually with the addition of *B. coagulans*, presumably to facilitate the growth of other denitrifying bacteria, such as *Gemmobacter*. This study demonstrates that advantageous colonization of *B. coagulans* can be achieved when combined with the findings discussed earlier. *B. coagulans* and the probiotic possess the capacity to generate antimicrobial agents that diminish the prevalence of harmful bacteria in the gut and augment the ratio of advantageous bacteria [66–68].

Fish have a sophisticated antioxidant regulatory system known as T-AOC, where SOD and CAT enzymes are commonly found and are essential to the protection of fish from oxidative stress [69–72]. An indication of lipid peroxidation is MDA content, with lower levels indicating better data [73–75]. Fish that were given diets containing *B. coagulans* had higher levels of SOD, CAT, and T-AOC activity in their livers. MDA in all the treatment groups decreased significantly. There was a positive effect demonstrated by the results of B. *coagulans*. There was an evaluation of the antioxidant capacity tested with *B. coagulans*, and the results aligned with Zhang et al. [6], it was reported that a basal diet supplemented with 5×10^9 CFU/kg of *Bacillus* spp. was indicated to significantly increase CAT and SOD levels and decrease MDA levels. According to Han et al. [57], it is possible to enhance the production of antioxidant enzymes and antioxidants, resulting in the effective removal of surplus free radicals and an improvement in antioxidant capacity.

Teleosts have a defense system composed largely of nonspecific immunity [40, 76, 77]. There is no doubt that inflammation is a key component of the innate immune response system, which is mediated primarily by cytokines [73]. Teleost fish can improve their immune response and anti-infection ability against pathogens by regulating cytokines [78]. Cytokines, namely, *IL-1β*, *IL-12*, *IL-10*, and *TGF-β*, are frequently employed to assess an organism's

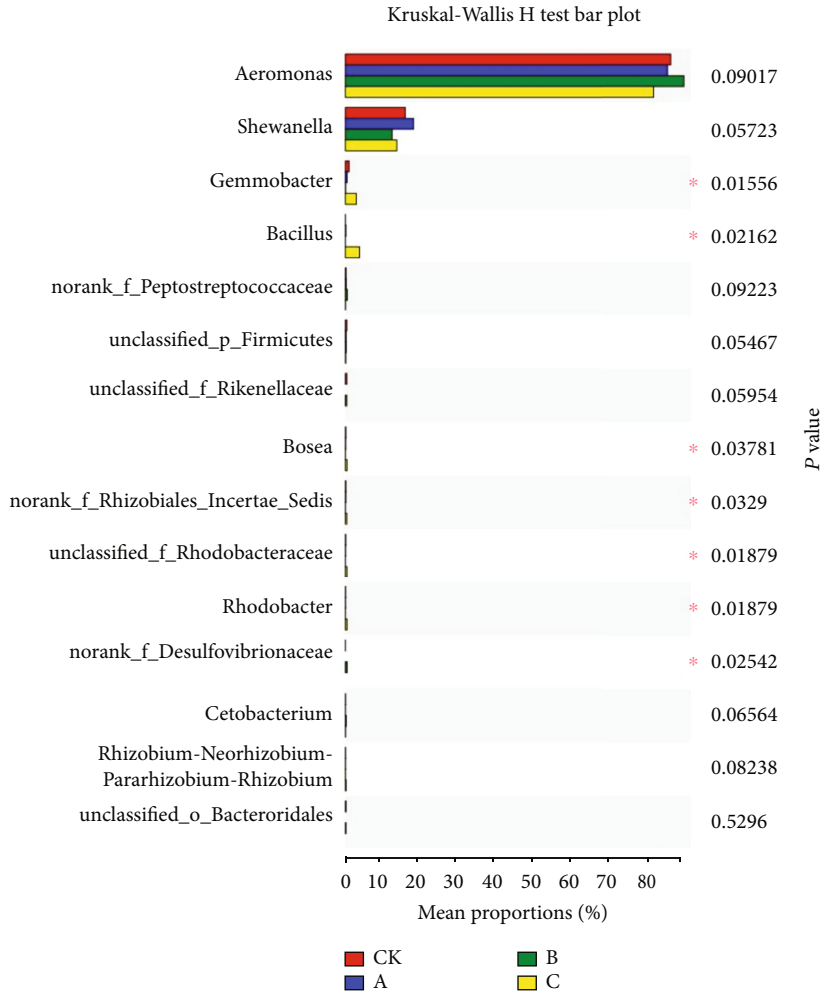

Figure 12: Species diversity analysis at genus level. Adding *Bacillus coagulans* to 1 g commercial diet: control (CK): 0 CFU/g; group A: 10^7 CFU/g; group B: 10^8 CFU/g; and group C: 10^9 CFU/g.

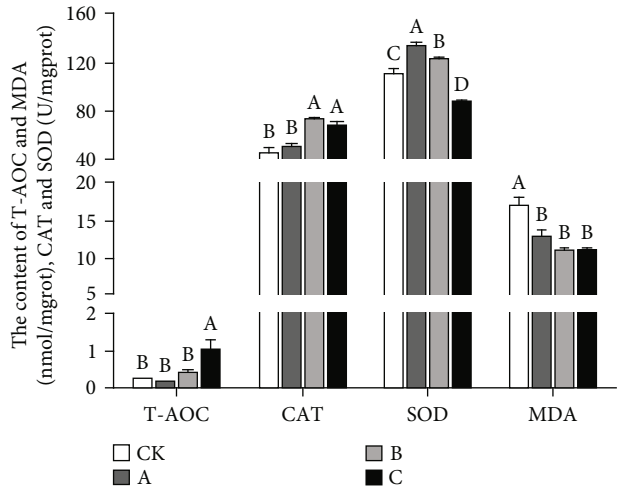

Figure 13: Total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and catalase (CAT) activity and malondialdehyde (MDA) concentration in the gut of common carp. The values marked with different letters are significantly different ($P < 0.05$).

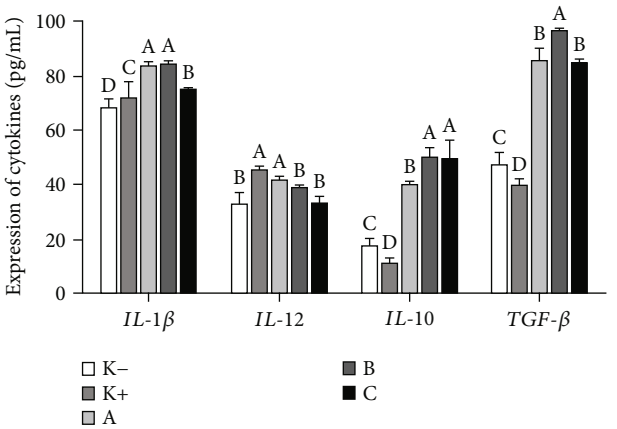

Figure 14: The expression level of cytokines (pg/mL). Negative control (K-); positive control (K+). Adding *B. coagulans* to 1 g commercial diet: control (CK), 0 CFU/g; group A, 10^7 CFU/g; group B, 10^8 CFU/g; and group C, 10^9 CFU/g. The values marked with different letters are significantly different ($P < 0.05$).

immune response, which can be further modulated by these cytokines [21]. As shown in this research, the introduction of A. *hydrophila* into a diet supplemented with *B. coagulans* increased the expression of *IL-1β*, *IL-12*, *IL-10*, and *TGF-β* in the serum of nonspecific immunity, which promotes fish's immunity. In addition, *B. coagulans* could induce nonspecific immunity, which promotes fish immunity. *B. coagulans* protect fish from invasive organisms. Standen et al. [79] found that tilapia was more responsive to probiotic supplementation, which is in line with our findings. Our study indicated that dietary supplementation of *B. coagulans* of common carp experiences effective prevention and alleviation of oxidative stress.

5. Conclusion

In conclusion, *B. coagulans* with GFP-tag had beneficial colonization in the intestine of common carp, which was maintained in the intestine for at least 14 days and more stable in the hindgut. A detailed analysis of the present study reveals that supplementing the diet of common carp with *B. coagulans* NRS 609 led to enhancements in growth performance, antioxidant capacity, intestinal barrier health, digestive enzyme activities, and disease resistance. The greater growth performance could potentially be attributed to boost digestive enzyme secretion and higher intestinal villi and muscular thickness in the probiotic-supplemented groups, promoting better feed digestibility and absorption. Additionally, the study indicated that *B. coagulans* positively influenced common carp's regulation of cytokines, which is an important component of the immune response, as well as modulating gut microbial diversity and composition, and enhancing liver antioxidant enzyme activity. A concentration of 10^7 CFU/g was found to be the optimal choice of *B. coagulans* in common carp diets, suggesting that the bacterial strain could serve as an effective and environmentally-friendly approach to managing fish aquaculture.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

We strictly comply with and reference the Henan Normal University Research Council's "Guide for the Care and Use of Laboratory Animals" and "Policy on Humane Care and Use of Laboratory Animals."

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jianxin Zhang and Mengyuan Huang contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31902361) and the Key Technology Research Project of Henan Province (222102110143).

References

[1] X. L. Chang, Y. L. Chen, J. C. Feng, M. Y. Huang, and J. X. Zhang, "Amelioration of Cd-induced bioaccumulation, oxidative stress and immune damage by probiotic _Bacillus coagulans_ in common carp (_Cyprinus carpio_ L.)," *Aquaculture Reports*, vol. 20, article 100678, 2021.

[2] M. A. O. Dawood, S. Koshio, S. E. Fadl et al., "The modulatory effect of mannanoligosaccharide on oxidative status, selected immune parameters and tolerance against low salinity stress in red sea bream (_Pagrus major_)," *Aquaculture Reports*, vol. 16, article 100278, 2020.

[3] E. Ahmadifar, M. S. Moghadam, M. A. O. Dawood, and S. H. Hoseinifar, "_Lactobacillus fermentum_ and/or ferulic acid improved the immune responses, antioxidative defence and resistance against _Aeromonas hydrophila_ in common carp (_Cyprinus carpio_) fingerlings," *Fish and Shellfish Immunology*, vol. 94, pp. 916–923, 2019.

[4] J. L. Wang, X. L. Meng, R. H. Lu et al., "Effects of _Rehmannia glutinosa_ on growth performance, immunological parameters and disease resistance to _Aeromonas hydrophila_ in common carp (_Cyprinus carpio_ L.)," *Aquaculture*, vol. 435, pp. 293–300, 2015.

[5] Z. G. Yi, J. Wang, T. Jiang, Q. Tang, and Y. Cheng, "Photocatalytic degradation of sulfamethazine in aqueous solution using ZnO with different morphologies," *Royal Society Open Science*, vol. 5, no. 4, article 171457, 2018.

[6] B. Zhang, H. R. Zhang, Y. Yu et al., "Effects of _Bacillus coagulans_ on growth performance, antioxidant capacity, immunity function, and gut health in broilers," *Poultry Science*, vol. 100, no. 6, article 101168, 2021.

[7] D. A. Beiwi and A. Al-Hisnawi, "Effect of *Bacillus subtilis* as probiotic on intestinal microbiota and growth performance of common carp (*Cyprinus carpio*)," in , Article ID 030004*AIP Conference Proceedings*, vol. 2290, Greece, 2020.

[8] W. Y. Mo, Z. Chen, H. M. Leung, and A. O. W. Leung, "Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks," *Environmental Science and Pollution Research*, vol. 24, no. 10, pp. 8978–8989, 2017.

[9] Q. Q. Zhou, Q. L. Xiao, Y. L. Zhang, X. L. Wang, Y. C. Xiao, and D. S. Shi, "Pig liver esterases PLE1 and PLE6: heterologous expression, hydrolysis of common antibiotics and pharmacological consequences," *Scientific Reports*, vol. 9, no. 1, p. 15564, 2019.

[10] M. A. Dawood, S. Koshio, and M. A. Esteban, "Beneficial roles of feed additives as immunostimulants in aquaculture: a review," *Reviews in Aquaculture*, vol. 10, no. 4, pp. 950–974, 2018.

[11] P. Jia, K. Cui, T. Ma et al., "Influence of dietary supplementation with _Bacillus licheniformis_ and _Saccharomyces cerevisiae_ as alternatives to monensin on growth performance, antioxidant, immunity, ruminal fermentation and microbial diversity of fattening lambs," *Scientific Reports*, vol. 8, no. 1, p. 16712, 2018.

[12] B. Vaseeharan and R. Thaya, "Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture," *Aquaculture International*, vol. 22, no. 3, pp. 1079–1091, 2014.

[13] A. R. Wang, C. Ran, Y. B. Wang et al., "Use of probiotics in aquaculture of China–a review of the past decade," *Fish and Shellfish Immunology*, vol. 86, pp. 734–755, 2019.

[14] L. R. Fitzpatrick, "Probiotics for the treatment of Clostridium difficile associated disease," *World Journal of Gastrointestinal Pathophysiology*, vol. 4, no. 3, pp. 47–52, 2013.

[15] M. A. O. Dawood, S. Koshio, M. M. Abdel-Daim, and H. V. Doan, "Probiotic application for sustainable aquaculture," *Reviews in Aquaculture*, vol. 11, no. 3, pp. 907–924, 2019.

[16] F. K. A. Kuebutornye, E. D. Abarike, and Y. Lu, "A review on the application of _Bacillus_ as probiotics in aquaculture," *Fish and Shellfish Immunology*, vol. 87, pp. 820–828, 2019.

[17] N. Akhter, B. Wu, A. M. Memon, and M. Mohsin, "Probiotics and prebiotics associated with aquaculture: a review," *Fish and Shellfish Immunology*, vol. 45, no. 2, pp. 733–741, 2015.

[18] E. Ringø, "Probiotics in shellfish aquaculture," *Aquaculture and Fisheries*, vol. 5, no. 1, pp. 1–27, 2020.

[19] K. Amoah, Q. C. Huang, B. P. Tan et al., "Dietary supplementation of probiotic _Bacillus coagulans_ ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, _Litopenaeus vannamei_," *Fish and Shellfish Immunology*, vol. 87, pp. 796–808, 2019.

[20] T. Manjula, "Growth promoting potential and colonization ability of probiotics (*Bacillus coagulans* and *Bacillus subtilis*) on the freshwater prawn," *Macrobrachium rosenbergii Post-Larvae*, vol. 2, pp. 007–018, 2018.

[21] R. Q. Fu, D. W. Chen, G. Tian et al., "Effect of dietary supplementation of _Bacillus coagulans_ or yeast hydrolysates on growth performance, antioxidant activity, cytokines and intestinal microflora of growing-finishing pigs," *Animal nutrition*, vol. 5, no. 4, pp. 366–372, 2019.

[22] S. B. Gu, L. N. Zhao, Y. Wu et al., "Potential probiotic attributes of a new strain of *Bacillus coagulans* CGMCC 9951 isolated from healthy piglet feces," *World Journal of Microbiology and Biotechnology*, vol. 31, no. 6, pp. 851–863, 2015.

[23] C. C. Lazado, C. M. A. Caipang, and E. G. Estante, "Prospects of host-associated microorganisms in fish and penaeids as probiotics with immunomodulatory functions," *Fish and Shellfish Immunology*, vol. 45, no. 1, pp. 2–12, 2015.

[24] J. Y. Yang, X. J. Guo, W. Guo, J. T. Lv, H. S. Yuan, and B. C. Zhu, "Screening and identification of a strain of *Bacillus* sp. producing organic acids and optimization of its spore production conditions," *Feed Industry*, vol. 36, pp. 44–51, 2015.

[25] X. L. Chang, M. R. Kang, Y. H. Shen et al., "_Bacillus coagulans_ SCC-19 maintains intestinal health in cadmium-exposed common carp (_Cyprinus carpio_ L.) by strengthening the gut barriers, relieving oxidative stress and modulating the intestinal microflora," *Ecotoxicology and Environmental Safety*, vol. 228, article 112977, 2021.

[26] Y. H. Zhou, Z. H. Zeng, Y. B. Xu et al., "Application of Bacillus coagulans in animal husbandry and its underlying mechanisms," *Animal*, vol. 10, no. 3, p. 454, 2020.

[27] G. N. Zhang, J. M. Zhang, and W. Gu, *The characteristics and research progress of Bacillus coagulans*, Feed and Animal Husbandry, 2013.

[28] C. G. M. Gahan and C. Hill, "Gastrointestinal phase of Listeria monocytogenes infection," *Journal of Applied Microbiology*, vol. 98, no. 6, pp. 1345–1353, 2005.

[29] A. J. H. Maathuis, D. Keller, and S. Farmer, "Survival and metabolic activity of the GanedenBC30 strain of *Bacillus coagulans* in a dynamic in vitro model of the stomach and small intestine," *Beneficial Microbes*, vol. 1, no. 1, pp. 31–36, 2010.

[30] W. H. Chu and C. P. Lu, "_In vivo_ fish models for visualizing _Aeromonas hydrophila_ invasion pathway using GFP as a biomarker," *Aquaculture*, vol. 277, no. 3-4, pp. 152–155, 2008.

[31] J. F. Yin, X. Y. Zhou, J. Q. Li, Y. H. Li, H. L. Hou, and W. H. Zhang, "A study on the survival dynamics of *Bacillus subtilis* in water using green fluorescent protein labelling Chinese," *Journal of Analytical Chemistry*, vol. 35, pp. 1405–1409, 2007.

[32] M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher, "Green fluorescent protein as a marker for gene expression," *Science*, vol. 263, no. 5148, pp. 802–805, 1994.

[33] R. H. Valdivia, A. E. Hromockyjm, D. Monack, L. Ramakrishnan, and S. Falkow, "Applications for green fluorescent protein (GFP) in the study of hostpathogen interactions," *Gene*, vol. 173, no. 1, pp. 47–52, 1996.

[34] L. N. Cao, L. F. Pan, L. Gong et al., "Interaction of a novel *Bacillus velezensis* (BvL03) against Aeromonas hydrophila in vitro and in vivo in grass carp," *Applied Microbiology Biotechnology*, vol. 103, no. 21-22, pp. 8987–8999, 2019.

[35] C. Y. Zhao, S. Yang, L. M. OuYang, and Y. H. Wang, "High osmolarity improves the electro-transformation efficiency of the gram-positive bacteria Bacillus coagulans p 4–102B," *Journal of Microbiology*, vol. 43, pp. 1388–1394, 2016.

[36] M. Ashida and T. Sasaki, "A target protease activity of serpins in insect hemolymph," *Insect Biochemistry and Molecular Biology*, vol. 24, no. 10, pp. 1037–1041, 1994.

[37] P. Bernfeld, "Amylases α and β," in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds., p. 149, Academic Press, USA, 1995.

[38] Y. Natalia, R. Hashim, A. Ali, and A. Chong, "Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue _Scleropages formosus_ (Osteoglossidae)," *Aquaculture*, vol. 233, no. 1-4, pp. 305–320, 2004.

[39] X. L. Chang, H. Li, J. C. Feng, Y. Y. Chen, G. X. Nie, and J. X. Zhang, "Effects of cadmium exposure on the composition and diversity of the intestinal microbial community of common carp (_Cyprinus carpio_ L.)," *Ecotoxicology and Environmental Safety*, vol. 171, pp. 92–98, 2019.

[40] J. C. Feng, X. L. Chang, Y. R. Zhang, X. Yan, J. X. Zhang, and G. X. Nie, "Effects of _Lactococcus lactis_ from _Cyprinus carpio_ L. as probiotics on growth performance, innate immune response and disease resistance against _Aeromonas hydrophila_," *Fish and Shellfish Immunololy*, vol. 93, pp. 73–81, 2019.

[41] J. N. Pu, D. W. Chen, G. Tian et al., "Effects of benzoic acid, _Bacillus_ coagulans and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets," *Animal Nutrition*, vol. 6, no. 2, pp. 152–159, 2020.

[42] B. Austin, L. F. Stuckey, P. A. W. Robertson, I. Effendi, and D. R. W. Griffith, "A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida*, *Vibrio anguillarum* and *Vibrio ordalii*," *Journal of Fish Diseases*, vol. 18, no. 1, pp. 93–96, 1995.

[43] L. F. Gibson, J. Woodworth, and A. M. George, "Probiotic activity of _Aeromonas media_ on the Pacific oyster,

Crassostrea gigas , when challenged with _Vibrio tubiashii_," *Aquaculture*, vol. 169, no. 1-2, pp. 111–120, 1998.

[44] J. H. Xu, S. Li, L. He, and S. J. Lv, "Survival dynamics and colonization of exogenous probiotic bacteria *Bacillus subtilis* in aquaculture water and intestine of zebra fish (*Danio rerio*)," *The Israeli journal of aquaculture Bamidgeh*, vol. 67, p. 1232, 2016.

[45] E. Ringo and F. J. Gatesoupe, "Lactic acid bacteria in fish: a review," *Aquaculture*, vol. 160, no. 3-4, pp. 177–203, 1998.

[46] F. M. Elshaghabee, N. Rokana, R. D. Gulhane, C. Sharma, and H. Panwar, "*Bacillus* as potential probiotics: status, concerns, and future perspectives," *Frontiers in Microbiology*, vol. 8, p. 1490, 2017.

[47] A. Khalique, D. Zeng, M. Shoaib, H. Wang, and X. Ni, "Probiotics mitigating subclinical necrotic enteritis (SNE) as potential alternatives to antibiotics in poultry," *AMB Express*, vol. 10, no. 1, pp. 538–542, 2020.

[48] F. Sadeghi, E. Ahmadifar, M. M. Shahriari, M. Ghiyasi, A. O. M. Dawood, and S. Yilmaz, "Lemon, Citrus aurantifolia, peel and Bacillus licheniformis protected common carp, Cyprinus carpio, from Aeromonas hydrophila infection by improving the humoral and skin mucosal immunity, and antioxidative responses," *Journal of the World Aquaculture Society*, vol. 52, pp. 124–137, 2020.

[49] M. A. Ramos, S. Batista, M. A. Pires et al., "Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia," *Animal An International Journal of Animal Bioscience*, vol. 11, no. 8, pp. 1259–1269, 2017.

[50] X. L. Gao, M. Zhang, X. Li, Y. Han, F. C. Wu, and Y. Liu, "Effects of a probiotic (Bacillus licheniformis) on the growth, immunity, and disease resistance of Haliotis discus hannai Ino," *Fish and Shellfish Immunology*, vol. 76, pp. 143–152, 2018.

[51] X. L. Gao, C. H. Ke, M. Zhang, X. Li, F. C. Wu, and Y. Liu, "*Effects of the probiotic _Bacillus amyloliquefaciens_ on the growth, immunity, and disease resistance of _Haliotis discus hannai_*," *Fish and Shellfish Immunology*, vol. 94, pp. 617–627, 2019.

[52] Y. J. Gao, H. J. Yang, Y. J. Liu et al., "*Effects of graded levels of threonine on growth performance, biochemical parameters and intestine morphology of juvenile grass carp _Ctenopharyngodon idella_*," *Aquaculture*, vol. 424-425, pp. 113–119, 2014.

[53] G. Huerta-Aguirre, K. M. Paredes-Ramos, M. P. Becerra-Amezcua, I. Hernández-Calderas, M. Matadamas-Guzman, and X. Guzmán-García, "Histopathological analysis of the intestine from Mugil cephalus on environment reference sites," in *Pollution of Water Bodies in Latin America*, L. Gómez-Oliván, Ed., pp. 319–328, Springer, Cham, 2019.

[54] H. M. R. Abdel-Latif, M. Abdel-Tawwab, A. F. Khafaga, and M. A. O. Dawood, "Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (_Cyprinus carpio_ L.) fingerlings," *Aquaculture*, vol. 526, article 735432, 2020.

[55] J. Romero, E. Ringø, and D. L. Merrifield, "The gut microbiota of fish," *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*, pp. 75–100, 2014.

[56] C. N. Zhang, X. F. Li, W. N. Xu et al., "Combined effects of dietary fructooligosaccharide *andBacillus licheniformison* growth performance, body composition, intestinal enzymes activities and gut histology of triangular bream (*Megalobrama terminalis*)," *Aquaculture Nutrition*, vol. 21, no. 5, pp. 755–766, 2015.

[57] B. Han, W. Q. Long, J. Y. He, Y. J. Liu, Y. Q. Si, and L. X. Tian, "Effects of dietary _Bacillus licheniformis_ on growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (_Oreochromis niloticus_) to challenge infections," *Fish and Shellfish Immunology*, vol. 46, no. 2, pp. 225–231, 2015.

[58] H. Zokaeifar, J. L. Balcázar, C. R. Saad et al., "*Effects of _Bacillus subtilis_ on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, _Litopenaeus vannamei_*," *Fish and Shellfish Immunology*, vol. 33, no. 4, pp. 683–689, 2012.

[59] N. Gobi, B. Vaseeharan, J. C. Chen et al., "*Dietary supplementation of probiotic _Bacillus licheniformis_ Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against _Aeromonas hydrophila_ in tilapia _Oreochromis mossambicus_*," *Fish and Shellfish Immunology*, vol. 74, pp. 501–508, 2018.

[60] S. P. Claus, H. Guillou, and S. Ellero-Simatos, "The gut microbiota: a major player in the toxicity of environmental pollutants?," *Npj biofilms and microbiomes*, vol. 2, no. 1, pp. 1–11, 2016.

[61] M. X. Byndloss and A. J. Bäumler, "The germ-organ theory of non-communicable diseases," *Nature Reviews Microbiology*, vol. 16, no. 2, pp. 103–110, 2018.

[62] S. Y. Miao, C. Z. Zhao, J. Y. Zhu, J. T. Hu, X. J. Dong, and L. S. Sun, "Dietary soybean meal affects intestinal homoeostasis by altering the microbiota, morphology and inflammatory cytokine gene expression in northern snakehead," *Scientific Reports*, vol. 8, no. 1, p. 113, 2018.

[63] J. F. Shan, *Effects of Bacillus Coagulans on Water Quality, Intestinal Microflora, Non-specific Immunity and Disease Resistance of Oriental River Prawn Macrobrachium Nipponense*, Journal of Dalian Ocean University, 2019.

[64] S. Y. Miao, W. L. Wan, J. T. Hu et al., "Dietary arachidonic acid affects the innate immunity, antioxidant capacities, intestinal health and microbiota in Chinese mitten crab (_Eriocheir sinensis_)," *Aquaculture*, vol. 548, article 737635, 2022.

[65] I. B. Saticioglu, F. Isbilir, O. Yavas et al., "Description of lens atrophy caused by _Shewanella_ sp. strain S-1, a putative novel species isolated from cultured rainbow trout," *Aquaculture*, vol. 541, article 736779, 2021.

[66] A. Das, S. Acharya, B. K. Behera et al., "Isolation, identification and characterization of _Klebsiella pneumoniae_ from infected farmed Indian Major Carp _Labeo rohita_ (Hamilton 1822) in West Bengal, India," *Aquaculture*, vol. 482, pp. 111–116, 2018.

[67] A. Daneshmand, H. Kermanshahi, M. H. Sekhavati et al., "Effects of cLFchimera peptide on intestinal morphology, integrity, microbiota, and immune cells in broiler chickens challenged with necrotic enteritis," *Scientific Reports*, vol. 10, no. 1, p. 17704, 2020.

[68] R. V. Oliveira, P. G. Peixoto, D. D. Ribeiro et al., "Klebsiella pneumoniae as a main cause of infection in Nishikigoi *Cyprinus carpio* (carp) by inadequate handing," *Brazilian Journal of Veterinary Pathology*, vol. 7, pp. 86–88, 2014.

[69] D. Gunapathy, R. Harikrishnan, B. A. Paray, M. K. Al-Sadoon, S. H. Hoseinifar, and C. Balasundaram, "*Comparative immunostimulatory effect of probiotics and prebiotics in _Channa punctatus_ against _Aphanomyces invadans_*," *Fish and Shellfish Immunology*, vol. 86, pp. 965–973, 2019.

[70] H. E. D. Mahmoud, A. A. Khairy, E. Salwa, A. O. D. Mahmoud, M. E. Enas, and A. P. Bilal, "Oxidative stress, hemato-

immunological, and intestinal morphometry changes induced by ochratoxin A in APRI rabbits and the protective role of probiotics," *Environmental Science and Pollution Research*, vol. 27, pp. 35439–35448, 2020.

[71] Y. Wang, Y. P. Wu, Y. Y. Wang et al., "Antioxidant properties of probiotic bacteria," *Nutrients*, vol. 9, no. 5, p. 521, 2017.

[72] Y. N. Wu, Y. Zhou, C. Lu et al., "Influence of butyrate loaded clinoptilolite dietary supplementation on growth performance, development of intestine and antioxidant capacity in broiler chickens," *PLoS One*, vol. 11, no. 4, article e0154410, 2016.

[73] M. T. Hasan, W. Je Jang, J. M. Lee et al., "Effects of immunostimulants, prebiotics, probiotics, synbiotics, and potentially immunoreactive feed additives on olive flounder (*Paralichthys olivaceus*): A review," *Reviews in Fisheries Science & Aquaculture*, vol. 27, no. 4, pp. 417–437, 2019.

[74] Y. Z. Li, Y. M. Yang, L. M. Song et al., "Effects of dietary supplementation of _Lactobacillus plantarum_ and _Bacillus subtilis_ on growth performance, survival, immune response, antioxidant capacity and digestive enzyme activity in olive flounder (_Paralichthys olivaceus_)," *Aquaculture and Fisheries*, vol. 6, no. 3, pp. 283–288, 2021.

[75] H. J. Zhang, Q. W. Ding, A. R. Wang et al., "Effects of dietary sodium acetate on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish: zebrafish as a model," *Aquaculture*, vol. 523, p. 735188, 2020.

[76] C. Li, Y. C. Ren, S. H. Jiang et al., "*Effects of dietary supplementation of four strains of lactic acid bacteria on growth, immune-related response and genes expression of the juvenile sea cucumber _Apostichopus japonicus_ Selenka*," *Fish and Shellfish Immunology*, vol. 74, pp. 69–75, 2018.

[77] Y. Sun, M. W. He, Z. J. Cao et al., "Effects of dietary administration of _Lactococcus lactis_ HNL12 on growth, innate immune response, and disease resistance of humpback grouper (_Cromileptes altivelis_)," *Fish and Shellfish Immunology*, vol. 82, pp. 296–303, 2018.

[78] J. C. Feng, L. H. Wang, L. X. Zhou, X. Yang, X. Zhao, and R. R. Martin, "Using in vitro immunomodulatory properties of lactic acid bacteria for selection of probiotics against salmonella infection in broiler chicks," *PLoS One*, vol. 11, no. 1, article e0147630, 2016.

[79] B. T. Standen, D. L. Peggs, M. D. Rawling et al., "*Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, _Oreochromis niloticus_*," *Fish and Shellfish Immunology*, vol. 49, pp. 427–435, 2016.