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Original Research Articles

Comparative analysis of the structural and compositional change of spotted sea bass (*Lateolabrax maculatus*) gut microflora following *Aeromonas veronii* infection and the effects of *Lactobacillus plantarum* on these changes

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Growing evidence suggests a close relationship between gut microbiota and infectious diseases. However, the specific role of gut microbiota in host-pathogen interactions during aquaculture-related infections remains poorly understood. This study investigated the diversity and composition of gut microbiota communities in *Aeromonas veronii*-infected *Lateolabrax maculatus* using high-throughput sequencing. The results revealed significant changes in the structure and composition of *L. maculatus* gut microbiota after *A. veronii* infection. Over time, Bacteroidetes and Firmicutes decreased significantly, while Proteobacteria increased significantly after *A. veronii* infection. Most intestinal bacteria showed a decline in abundance over time, with probiotics (such as *Lactobacillus*) experiencing a significant decrease and pathogens (such as *Aeromonas*) showing a significant increase. Conversely, no differences were observed in the structure and composition of gut microbiota between healthy *L. maculatus* and those infected with *A. veronii* after treatment with *Lactobacillus plantarum*; no changes in relative abundances of other bacterial phyla or genera except for *Aeromonas*. Furthermore, intestinal flora's structural diversity and composition differed significantly from untreated *L. maculatus* infected with *A. veronii*. These findings suggest alterations in the structure and composition of gut microbiota following *A. veronii* infection. *L. plantarum* can maintain a dynamic balance within the intestinal flora, reducing the potential risk of pathogen infections.

INTRODUCTION

The global population is growing rapidly, leading to an increased demand for food.[1] Ensuring that food provision is both safe and sustainable is of paramount importance. Over the past few decades, aquaculture has emerged as one of the fastest-growing animal food sources, owing to its rapid expansion and intensive development. It plays an increasingly important role in the global food supply.[2-4] Infectious diseases caused by viruses, bacteria, and other pathogens are common occurrences in the aquaculture industry, resulting in substantial economic losses and significantly impacting its sustainable development.[5] As aquaculture plays a vital role in global food security, understanding and managing the factors influencing fish health, such as gut microbiota dynamics during infections, contribute to ensuring a stable and secure food supply.

Diseases caused by bacterial infections, such as *Aeromonas. spp*, are a major cause of high mortality in farmed fish. These infections can lead to bacterial hemorrhagic septicemia (BHS),[6] exercise *Aeromonas* septicemia (MAS)[7] and epidemic ulcer syndrome (EUS).[8] Among these infections, *Aeromonas veronii* (*A. veronii*), identified as an opportunistic pathogen,[9,10] has demonstrated its ability to

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infect a diverse range of fish species, such as cyprinid fish,[11] Largemouth bass (*Micropterus salmoides*),[12] freshwater dark sleeper (*Odontobutis potamophila*),[13] Nile tilapia (*Oreochromis niloticus*),[14] and crucian carp (*Carassius carassius*),[15] among others. Recent research has indicated a potential association between *A. veronii* and alterations in intestinal flora.[16]

The gut microbiota comprises various microorganisms, including probiotics, pathogens, and neutral bacteria.[17,18] These microorganisms play important roles in regulating the host's physiology, immunity, and nutrition.[19-21] Previous studies have demonstrated a strong connection between alterations in intestinal flora and numerous diseases, including diarrhea, obesity, diabetes, and cancer.[22-25] Several studies have also revealed that pathogen infections can alter the composition of fish intestinal microflora.[26-28] The intestinal tract may also serve as a potential source or route for pathogenic infections.[29,30] Simultaneously, Recent research has highlighted the significant role of probiotics as alternatives to antibiotics for disease control, which have shown substantial effects in modulating gut microbiota, promoting host growth and metabolism, and inhibiting pathogens.[31-33] *Lactobacillus plantarum* (*L. plantarum*), a Gram-positive lactic acid bacterium, has demonstrated its potential as a probiotic, particularly in suppressing pathogenic bacteria and regulating host gut microbiota.[34] Studies have shown that including *L. plantarum* in feed can enhance the growth of Nile tilapia and increase resistance to *Aeromonas hydrophila* infection.[35] In the large yellow croaker (*Larimichthys crocea*), *L. plantarum* has exhibited significant antimicrobial activity against several aquaculture-related pathogens.[36] Moreover, bacteriocins produced by *L. plantarum* effectively inhibit the growth of *Staphylococcus aureus*, *Listeria monocytogenes* and other bacteria.[37] These findings demonstrate the potential of L. plantarum as a probiotic in aquaculture, offering a viable alternative to antibiotics for disease prevention and control.

Lateolabrax maculatus (*L. maculatus*) is widely distributed in East Asia, extending to the border between China and Vietnam in the south to the southeast coast of South Korea in the north.[38] This species holds significant economic importance and has gained prominence as one of the most promising breeds owing to the rapid growth of the *L. maculatus* culture industry.[39] However, *L. maculatus* is susceptible to *Aeromonas* disease caused by *A. veronii*.[40] Currently, most research on fish disease resistance mechanisms mainly focuses on physiological and transcriptional aspects post-infection, with limited exploration of the role of intestinal microorganisms in disease resistance. There have been limited studies on the composition and structure of the intestinal microflora of *L. maculatus* during bacterial infection. Additionally, the impact of *L. plantarum* on the gut microbiota of *L. maculatus* remains unexplored. Therefore, this study aimed to investigate the composition and differences in the intestinal flora of healthy and *A. veronii*-infected *L. maculatus,* evaluate the microbiota changes caused by the disease, and assess the effect of *L. plantarum* on the gut microbiota of *L. maculatus* during bacterial infection. Based on the previous information, we harvested intestinal tissue samples from healthy *L. maculatus* at various time points after injection of *A. veronii,* preceded by the pretreatment with *L. plantarum*. This study holds the potential to provide theoretical insights into enhancing the prevention and control of bacterial infections and the development of probiotics within large-scale, high-density, and intensive cultures of *L. maculatus*.

MATERIALS AND METHODS

ETHICS STATEMENT

L. maculatus is neither an endangered nor a protected species, and conducting experiments with this species in China does not require permission. The animal experiment was approved by the Animal Ethics Committee of the Chinese Academy of Fishery Sciences (Approval No. 2011AA1004020012).

EXPERIMENTAL ANIMALS, BACTERIAL, AND SAMPLE COLLECTION

Healthy *L. maculatus* specimens were sourced from the Zhuhai Experimental Base of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (Guangzhou, China). Prior to infection, *L. maculatus* (65 ± 3 g) were randomly distributed into two separate culture buckets (500 L) with 30 fish in each bucket. They were maintained in aerated freshwater at 29 ± 1 °C, with one-third of the water being replaced daily.

Feeding was stopped 24 h before the start of the experiment. In the bacterial challenge experiment, the entire intestinal tissues of three fish were initially extracted from each of the two culture buckets. Subsequently, the remaining fish in both culture buckets were injected intraperitoneally with *A. veronii* at a dose of 8.5×10^8 CFU/g. The entire intestinal tissues were collected from *L. maculatus* at two distinct time points (24 and 48 h) post-injection (three biological replicates at each time point). All collected samples were frozen in liquid nitrogen and stored at -80 °C for further analysis. The *A. veronii* colonies utilized in the challenge tests were preserved in our laboratory. The *L. plantarum* used in the experiment is a commercially available strain commonly used as a dietary supplement.

DNA EXTRACTION

Microbial DNA was extracted using the HiPure Soil DNA Kit or HiPure Stool DNA Kit (Magen, Guangzhou, China) according to the instructions of the manufacturer. The V3–V4 region of the 16S rDNA target, part of the ribosomal RNA gene, was amplified by polymerase chain reaction (PCR) (95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 7 min) using primers 341F (CCTACGG GNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT). The PCR reaction mixture consisted of 50 µL, containing 10 µL of 5 × Q5@ Reaction Buffer, 10 µL of 5 × Q5@ High GC Enhancer, 1.5 µL of 2.5 mM deoxynucleotide triphosphates (dNTPs), 1.5 µL of each primer (10 µM), 0.2 µL of Q5@

High-Fidelity DNA Polymerase, and 50 ng of template DNA. All PCR reagents were purchased from New England Biolabs (USA). Amplicons were extracted from 2% agarose gels, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the instruction of the manufacturer, and quantified using the ABI StepOnePlus Real-Time PCR System (Life Technologies, USA). The purified amplicons were combined in equimolar proportions and subjected to paired-end sequenced (PE250) on an Illumina 2500 platform according to standard protocols.

BIOINFORMATICS ANALYSIS

After sequencing, raw reads were filtered using FASTP[41] (version 0.18.0) to obtain high-quality clean reads. Subsequently, clean paired-end reads were merged into raw tags using FLASH[42] (version 1.2.11) designed for accurate merging based on overlap analysis. Noisy sequences were removed to obtain high-quality clean tags using specific filtering conditions.[43] Utilizing the UPARSE pipeline[44] (version 9.2.64), the clean tags were clustered into operational taxonomic units (OTUs) with a similarity threshold of ⩾ 97%. All chimeric tags were removed using the UCHIME algorithm.[45] Representative sequences for each OTU were selected based on the highest abundance tag sequence. The representative OTU sequences were classified using a naive Bayesian model in the RDP classifier[46] (version 2.2) based on the SILVA database[47] (version 132), with a confidence threshold of 0.8. Abundance statistics for each taxonomy were visualized using Krona[48] (version 2.6). Community composition was visualized using stacked bar plots in R with the ggplot2 package[49] (version 2.2.1), and species abundance heatmaps were created using the pheatmap package[50] (version 1.0.12) in the R project. Venn diagrams for group comparisons were generated using the VennDiagram package[51] (version 1.6.16) in R. Alpha diversity metrics, including Chao1, Shannon, Simpson, and Good's coverage, were computed to assess species richness and evenness within microbial communities using the vegan package in QIIME[52] (version 1.9.1). including Adonis (PERMANOVA) and Anosim tests, were performed using the vegan package in R[53] (version 2.5.3) and plotted in R project ggplot2 package[49] (version 2.2.1). Statistical analysis of Adonis (also called Permanova) and Anosim test was calculated in R project Vegan package[53] (version 2.5.3). Diversity indices and bacterial abundances among different groups were compared using one-way ANOVA. Statistical significance was set at $P < 0.05$.

RESULTS

SEQUENCES ANALYSES

High-throughput sequencing was used to analyze sample sequences. After data optimization, 303,793 effective sequences were obtained from the 18 intestinal samples of *L. maculate* (Table. 1). Based on 97% sequence identity, the effective sequences were divided into 2,845 OUTs. The number of OTUs per sample varied, ranging from 161 to 760 (Table 1). Moreover, in the infection groups (V0, V24, and V48), 69 common OTUs were identified, while in the infection groups after pretreatment with *L. plantarum* (LV0, LV24, and LV48), 203 common OTUs were observed (Fig. 1 A and B). There were 51 common OTUs among the six different small groups (Fig. 1C). The saturation observed in the Shannon, Simpson, and rank abundance curves suggests that the sequencing depth, abundance, and homogeneity adequately capture gut microbiota diversity in the samples (Fig. 2A–C).

ANALYSIS OF THE DIVERSITY OF THE INTESTINAL MICROBIAL COMMUNITY

To assess the alpha diversity of gut microbial communities in various treatment groups and time points, Chao1, Shannon, and Good's coverage indices were used. The Good's coverage estimates across all samples ranged from 99.74% to 99.96%, indicating exceptional coverage (Table 2). Figure 3A presents the mean value of the Chao1 index, which was significantly higher in the V0 group than in the V24 ($p < 0.01$) and V48 groups ($p < 0.01$), indicating a significant difference in intestinal flora abundance between the V0 group and the other two groups. Similarly, the mean Chao 1 index was significantly higher in the LV24 group than in the V24 group ($p < 0.01$), indicating a significant difference in gut microbe abundance between these two groups. Furthermore, the mean Chao1 index was significantly higher in the LV48 group than in the V48 group ($p < 0.01$). Additionally, the mean Shannon's index was significantly higher in the V0 group than in the V24 and V48 groups ($p < 0.05$). This indicated a significant difference in the homogeneity of the gut microbes between the V0 group and the other two groups. The Shannon index was significantly lower in the V24 group than in the LV24 group ($p < 0.05$). A similar significant difference was observed in the V48 group compared with the LV48 group ($p < 0.05$) (Fig. 3B). However, the LV24 and LV48 groups showed large dispersion, and PcoA indicated a significant difference in the gut microbiota composition across all six groups (Adonis, $R2 = 0.569$, $p < 0.001$), with a 53.39% distance in PCo1. Notably, the LV24 and LV48 groups formed a cluster in PCo1, the V24 and V48 groups clustered together in PCo1, and the V0 group clustered with the LV0 group (Fig. 4A–B).

COMPOSITION ANALYSIS OF THE GUT MICROBIOTA COMMUNITY STRUCTURE AT THE PHYLUM LEVEL IN DIFFERENT GROUPS

The gut microbiota community structures of the different treatments were evaluated at the phylum level based on the sampling time. Among the 27 bacterial phyla identified, with a few unclassified OTUs, the dominant phyla in the gut microbiota of the V0 group (healthy fish) were Firmicutes (58.98%), Bacteroidetes (18.35%), Proteobacteria (9.92%), and Actinobacteria (5.72%) (Fig. 5A). The relative abundance of Proteobacteria increased significantly at 24 h ($p < 0.01$), whereas the relative abundances of Firmicutes ($p < 0.001$) and Bacteroidetes ($p < 0.001$) decreased significantly (Fig. 6A–C). In the *L. plantarum* pretreatment group, the dominant phyla in the gut microbiota of the

Table 1. Sequencing data for each sample

Sample	*Raw Reads*	*Effective Tags*	*OTUs*
V-0-1	130356	105749	595
V-0-2	133764	110239	636
V-0-3	123406	103373	621
V-24-1	136024	117026	174
V-24-2	124077	110901	208
V-24-3	122762	118196	349
V-48-1	129952	114606	193
V-48-2	123784	111114	283
V-48-3	132044	113303	161
LV-0-1	121079	99948	687
LV-0-2	137628	111702	605
LV-0-3	127880	104945	638
LV-24-1	136533	113588	624
LV-24-2	122326	99922	591
LV-24-3	122861	107341	682
LV-48-1	129016	124583	392
LV-48-2	124858	87536	559
LV-48-3	125198	101232	760

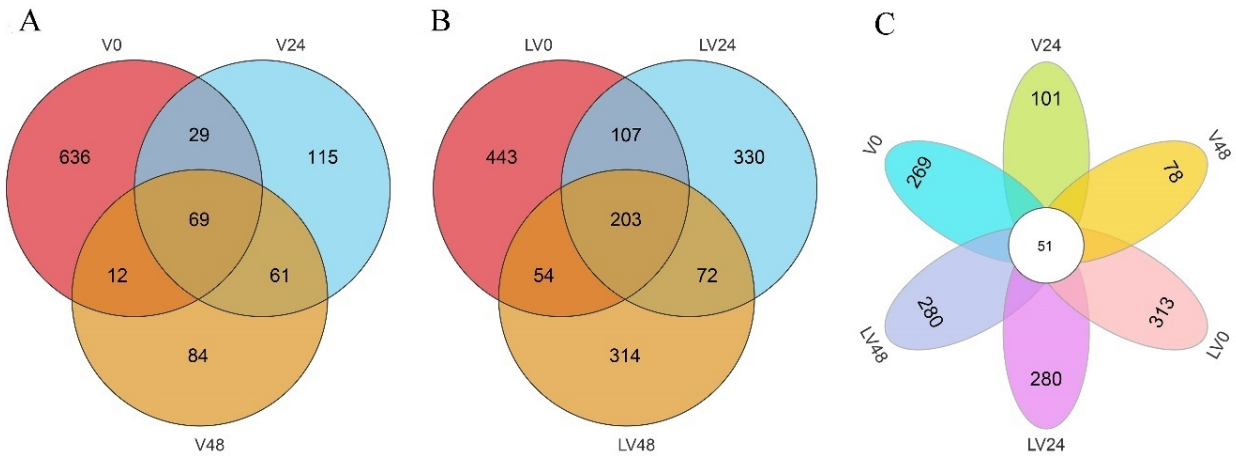

Figure 1. Venn diagrams depicting the composition of operational taxonomic units (OTUs).

A: Venn diagrams comparing the V0, V24, and V48 groups; B: Venn diagrams comparing the LV0, LV24, and LV48 groups; C: Venn diagrams comparing the six different groups. V0 represents untreated healthy fish, V24 represents 24 h after *A. veronii* infection, V48 represents 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A.veronii* infection under *L. plantarum* pretreatment.

LV0 group (healthy fish) were Firmicutes (55.61%), Bacteroidetes (19.84%), Proteobacteria (12.31%), and Actinobacteria (6.43%) (Fig. 5A). These were similar to the composition of the V0 group; however, their relative abundances differed. The relative abundances of Proteobacteria, Firmicutes, and Bacteroidetes remained stable over time; however, a notable contrast was observed in the untreated diseased fish. Specifically, the untreated diseased fish displayed a higher relative abundance of Proteobacteria, while the relative abundances of Firmicutes ($p < 0.001$) and Bacteroidetes ($p < 0.01$) were significantly lower compared with those in the diseased fish in *L. plantarum* pretreatment group (Fig. 6A–C).

The top 20 bacterial genera in each group are shown in the abundance heatmap (Fig. 5B). The results from Fig. 5B and C indicate that the dominant flora in both healthy fish and those treated with *L. plantarum* were the same, although the relative abundances differed. Over time, apart from a few bacterial genera (mostly pathogenic bacteria), the relative abundance of bacterial genera in the untreated diseased fish significantly decreased than that in healthy fish. Most of the bacterial genera in the untreated diseased fish exhibited a downward trend; however, the overall

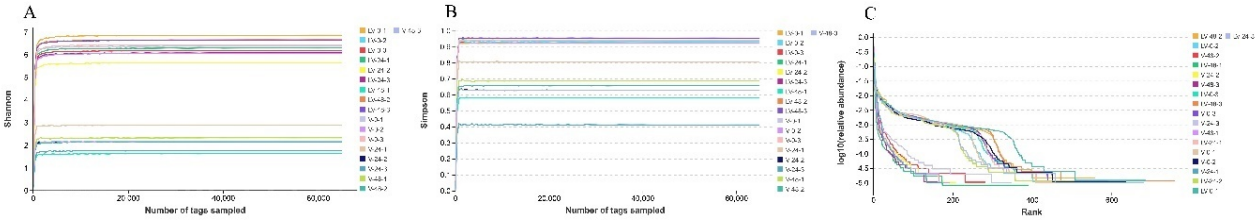

Figure 2. Feasibility analysis of sequencing data.

Rarefaction curves of Shannon(A), Simpson(B), and the rank abundance curve (C) were used to evaluate the sequencing depth, abundance, and homogeneity for each sample; each curve indicates a sample. V0 represents untreated healthy fish, V24 represents fish 24 h after *A. veronii* infection; V48 represents fish 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A. veronii* infection under *L. plantarum* pretreatment.

Table 2. Alpha diversity indices of gut microbiota in each group

Group	*Chao 1*	*Shannon*	*Goods coverage*
V0	819.6748	6.294777	0.997923542
V24	260.8141	2.266413	0.999530325
V48	224.686	2.212665	0.999601221
LV0	879.8382	6.374039	0.997751128
LV24	944.9923	6.006303	0.997559568
LV48	908.4409	4.979198	0.997446116

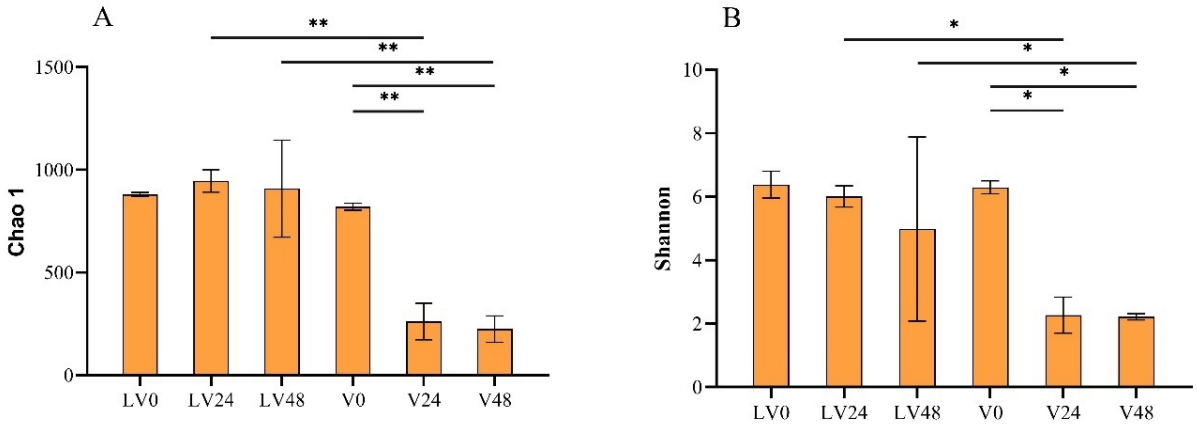

Figure 3. Comparative analysis of the alpha diversity indices of gut microbiota in different groups.

Alpha diversity of the gut microbiota was assessed using Chao1 (A) and Shannon (B). Statistical significance is denoted by *, $p < 0.05$; **, $p < 0.01$. V0 represents untreated healthy fish, V24 represents fish 24 h after *A. veronii* infection; V48 represents fish 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A. veronii* infection under *L. plantarum* pretreatment.

downward trend was lower than that in the diseased fish after *L. plantarum* treatment. Among them, the relative abundance of *Aeromonas* significantly increased at 24 h ($p < 0.001$) and decreased at 48 h ($p < 0.01$) in both untreated infected fish and *L. plantarum*-treated fish. The relative abundance of *Aeromonas* was significantly lower in the LV48 group than in the V48 group ($p < 0.001$) (Fig. 6D). For *Vibrio,* the relative abundance increased significantly over time in untreated infected fish ($p < 0.05$) compared with healthy fish, however, there was no significant change in infected fish treated with *L. plantarum* (Fig. 6E). Regarding *Acinetobacter*, the relative abundance in untreated diseased fish increased significantly increased at 24 h ($p < 0.001$) and decreased at 48 h ($p < 0.001$), while that of *Acinetobacter* in diseased fish treated with *L. plantarum* showed no significant change (Fig. 6F). The relative abundance of *Lactobacillus* and *Bifidobacterium* in untreated diseased fish significantly decreased over time than that in healthy fish ($p < 0.05$), but there was no significant difference in the relative abundance of *Lactobacillus* and *Bifidobacterium* in *L.*

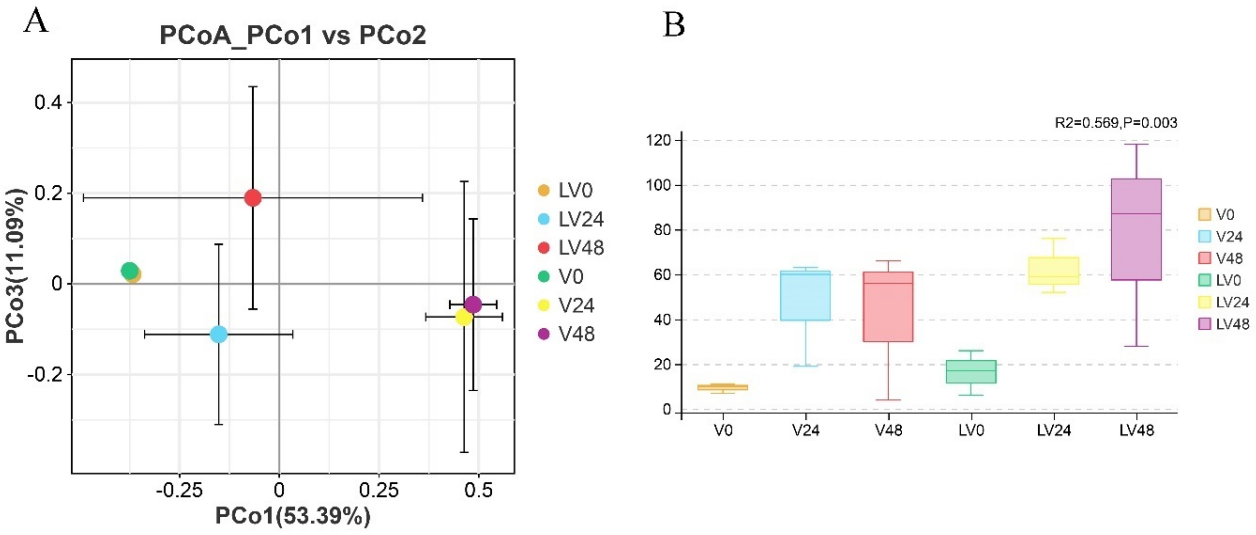

Figure 4. Comparative analysis of beta diversity indices of gut microbiota in different groups.

A: PCoA analysis of enteric microbes in different groups. B: Adonis (permanova) test in different groups. V0 represents untreated healthy fish, V24 represents fish 24 h after *A. veronii* infection; V48 represents fish 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A. veronii* infection under *L. plantarum* pretreatment.

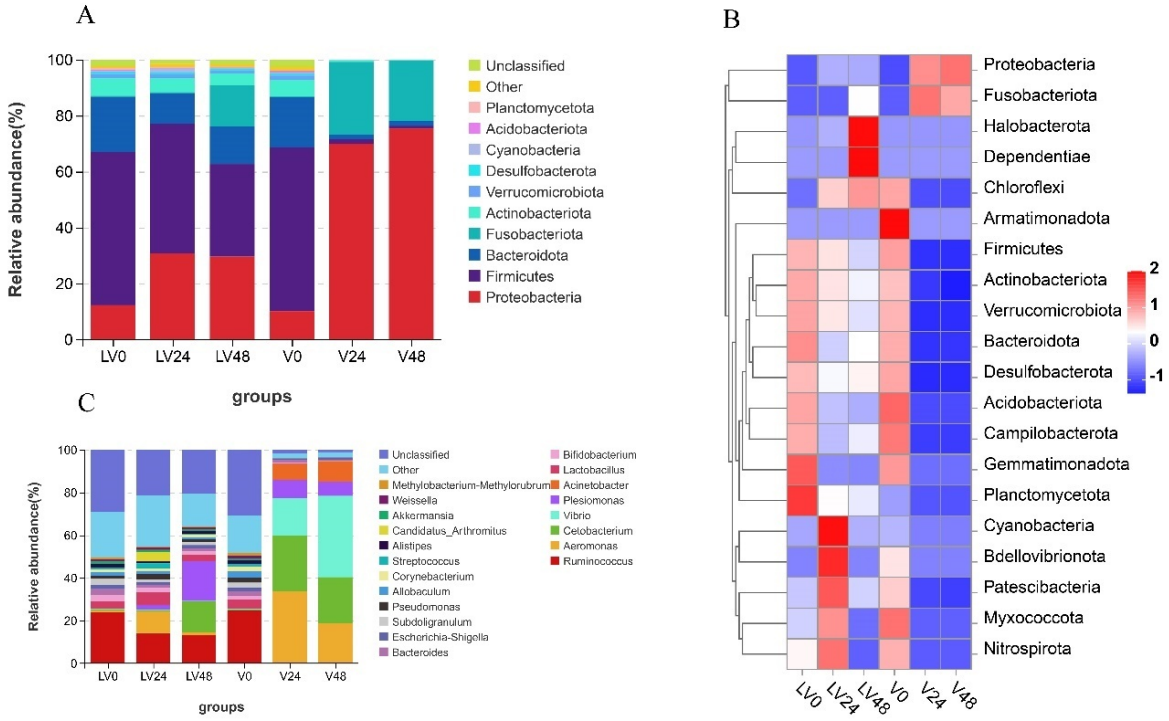

Figure 5. Relative abundance of the gut microbial composition of different groups.

A: Top 10 dominant phylum of the *L. maculatus* enteric microbiota composition, with the remaining phyla combined into the category "Others"; B: Top 20 primary genera of the *L. maculatus* enteric microbiota composition, with the remaining genera merged into the category "Others"; C: Heatmap illustrating the top 20 most abundant bacterial genera of bacterial genus level in each group. Bacterial genera and groups were clustered using the average algorithm, and the color blocks represent the relative abundance of each genus. V0 represents untreated healthy fish, V24 represents fish 24 h after *A. veronii* infection; V48 represents fish 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A. veronii* infection under *L. plantarum* pretreatment.

plantarum-treated diseased fish (Fig. 6G and H). The relative abundances of *Vibrio*, *Acinetobacter*, *Lactobacillus,* and *Bifidobacterium* in untreated diseased fish were significantly different from those in *L. plantarum*-treated diseased fish ($p < 0.05$) (Fig. 6E–H).

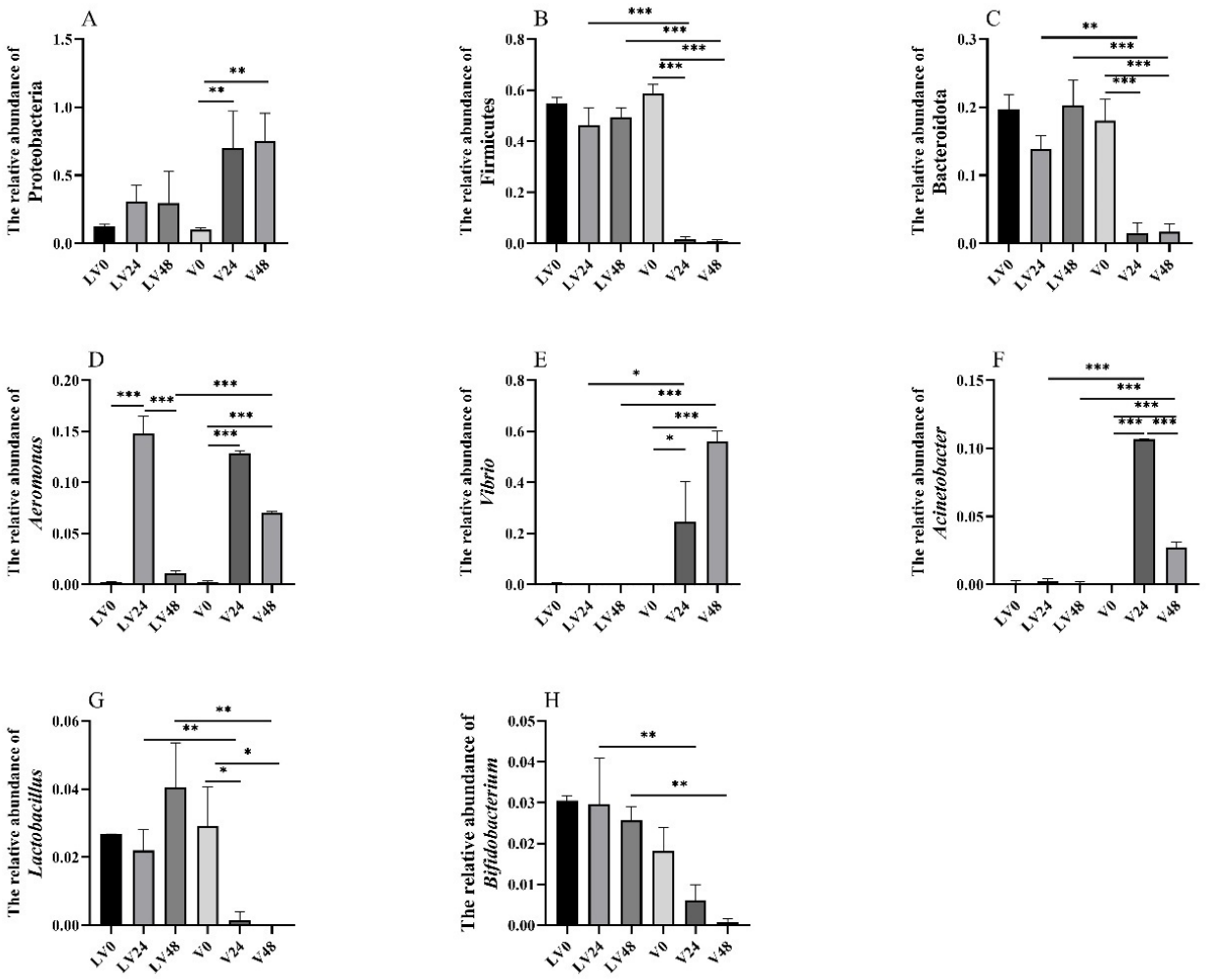

Figure 6. Differences in intestinal bacteria abundance were evaluated among each group.

A–C: Variations in phylum abundance among different groups; D–H: Disparities in genus abundance among each group. V0 represents untreated healthy fish, V24 represents fish 24 h after *A. veronii* infection; V48 represents fish 48 h after *A. veronii* infection; LV0 represents healthy fish with *L. plantarum* pretreatment, LV24 represents fish 24 h after *A. veronii* infection under *L. plantarum* pretreatment, and LV48 represents fish 48 h after *A. veronii* infection under *L. plantarum* pretreatment. The results were evaluated using one-way ANOVA. All data are presented as mean ± SD. Statistical significance is denoted by **P*<0.05, ***P*<0.01, ****P*<0.001.

DISCUSSION

Aeromonas spp. have consistently posed significant threats in aquaculture, causing infectious diseases that result in significant economic losses globally.[54] In recent years, the role of the gut microbiota in host disease resistance has become a frontier of research. Many studies have demonstrated the connection between various diseases and alterations in the intestinal microflora.[32,55,56] The gut microbiota plays a crucial role in preventing and treating diseases by regulating the intestinal barrier and environment, thereby reducing the invasion and colonization of pathogens. Given the growing threat posed by infectious pathogen invasion in the fish culture industry, it is essential to explore the relationship between pathogen invasion and the diversity of the intestinal flora. Such exploration is vital for understanding how intestinal microorganisms affect host health and for developing effective measures to prevent or treat diseases. Moreover, probiotics have been demonstrated to stimulate host growth and development, boost host immunity, and potentially replace antibiotics.[57] Using high-throughput sequencing, this study compared and analyzed the diversity and dynamic changes in the intestinal flora of *L. maculatus* infected with *A. veronii* and *L. maculatus* infected with *A. veronii* after pretreatment with *L. plantarum*. This study aimed to investigate the effects of *A. veronii* infection on the intestinal flora of *L. maculatus* and the modulation of the intestinal flora of *L. maculatus* by *L. plantarum*.

The Chao 1 index, Shannon index, and PCoA analyses revealed distinct patterns in this study. The untreated infected group exhibited a significant decrease in diversity over time. In contrast, the diversity of the infected group with *L. plantarum* pretreatment remained relatively stable over time, and the difference in diversity between the two groups was significant. These findings suggest that *A. veronii* infection reduces the abundance and evenness of microbial diversity in *L. maculatus*, while *L. plantarum* contributes to the preservation of microbial diversity. Previous

research has indicated that a higher diversity of gut microbes is advantageous for maintaining dynamic intestinal balance and function.[58]

Maintaining the "microbial balance" in the intestinal tract is vital to health. When analyzing the gut microbiota composition in *L. maculatus*, Firmicutes, Bacteroidetes, and Proteobacteria emerged as the dominant phyla, aligning with previous findings on fish intestinal flora.[59] Proteobacteria constituted the largest phylum in infected fish, encompassing well-studied pathogens and indicating dysbiosis in the gut microbiota.[60,61] The increase in the relative abundance of Proteobacteria may contribute to the development of inflammatory bowel disease.[62] These findings show that a higher Proteobacteria abundance heightens the susceptibility of fish to pathogenic infections. Additionally, Bacteroidetes was also a dominant phylum in the intestinal flora of healthy fish. Studies have shown that Bacteroides is related to the intestinal immune response,[63,64] and a higher abundance of Bacteroidetes helps to improve host immunity and contributes to the dynamic balance of gut microbiota.[65] In our study, compared with healthy fish, infected fish exhibited an increase in Proteobacteria abundance and a decrease in Bacteroidetes abundance over time. This suggests an imbalance in the intestinal flora of *A. veronii*-infected *L. maculatus*. In contrast, diseased fish treated with *L. plantarum* showed a lower abundance of Proteobacteria and a higher abundance of Bacteroidetes than those in untreated diseased fish. This indicates that *L. plantarum* treatment is beneficial in reducing infection risk and maintaining intestinal flora balance.

At the genus level, *A. veronii* infection increased the abundance of some pathogenic bacteria and reduced the abundance of many beneficial bacteria compared to healthy fish. This disturbance in intestinal flora's environmental balance may reduce the intestinal tract's resistance to pathogenic bacteria. In contrast, fish pretreated with *L. plantarum* exhibited a more stable intestinal flora, highlighting the role of *L. plantarum* in preserving microbial diversity during prevention and antibacterial treatment. Comparatively, untreated diseased fish displayed higher *Aeromonas*, *Vibrio*, and *Acinetobacter* levels and lower *Lactobacillus* and *Bifidobacterium* levels. While the *Aeromonas* content was higher in diseased fish with *L. plantarum* pretreatment than in healthy fish, there were no significant changes in *Vibrio*, *Acinetobacter*, *Lactobacillus,* and *Bifidobacterium*. Additionally, the *Vibrio*, *Acinetobacter*, *Lactobacillus,* and *Bifidobacterium* levels in untreated diseased fish were lower than those in diseased fish with *L. plantarum* pretreatment. Changes in *Aeromonas* may directly result from *A. veronii* infection, as indicated by a similar study.[66] Additionally, *Vibrio*, an opportunistic pathogen prevalent in freshwater and seawater, is a leading cause of *Vibrio* disease in aquaculture,[67] presenting symptoms such as fish septicemia and gastroenteritis, among other symptoms.[68] *Acinetobacter*, identified as a fish pathogen, poses a new threat to the aquaculture industry, leading to severe septicemia outbreaks in fish farms.[69] An imbalance in the intestinal microbial structure may have caused the increase in relative abundance of *Vibrio* and *Acinetobacter*. *Lactobacillus* and *Bifidobacterium* are probiotic bacteria. *Lactobacillus* promotes host growth and reproduction, improve immunity, and disease resistance,[70-72] while *Bifidobacterium* induces better growth of trout seedlings and increase digestion and nutritional utilization.[73] In addition, CpG oligodeoxynucleotides (ODNs) from *Bifidobacterium* may serve as immunostimulatory modulators of the immune response.[74] LAB combinations (*Lactobacillus* and *Bifidobacterium*) can improve the host's growth performance and intestinal health and reduce the risk of pathogenic infections.[75] Therefore, it is speculated that *A. veronii* infects the intestine and suppresses some beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*. This suppression could create an environment where *Aeromonas* and other pathogenic bacteria occupy more niches, ultimately disrupting the balance of intestinal flora. Treatment with *L. plantarum* helps maintain the dynamic balance of intestinal flora and potentially protects sea bass.

In conclusion, this study investigates the composition and differences in the gut microbiota of healthy and *A. veronii*-infected *L. maculatus* to assess the microbial alterations caused by the infection and evaluate the influence of *L. plantarum* during the bacterial infection process. The findings indicate that *A. veronii* infection disrupts the gut microbiota equilibrium in *L. maculatus*. *L. plantarum* may stabilize the gut microbiota, thereby providing protection against *A. veronii* infection. These results offer theoretical support for developing strategies to prevent bacterial infections and promote the use of probiotics in the intensive, high-density aquaculture of *L. maculatus*.

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AUTHORS' CONTRIBUTION – CREDIT TAXONOMY

Methodology: Changhong Lin (Lead). Formal Analysis: Changhong Lin (Equal), Lihua Qiu (Equal), Bo Zhang (Equal). Investigation: Changhong Lin (Equal), Lihua Qiu (Equal), Bo Zhang (Equal). Writing – original draft: Changhong Lin (Lead). Resources: Pengfei Wang (Equal), Bo Zhang (Equal). Writing – review & editing: Lulu Yan (Equal). Conceptualization: Chao Zhao (Lead). Funding acquisition: Chao Zhao (Lead). Supervision: Chao Zhao (Lead).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

RESEARCH INVOLVING ANIMALS

The animal experiment was approved by the Animal Ethics Committee of the Chinese Academy of Fishery Sciences (Approval No. 2011AA1004020012).

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

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The data that has been used is confidential.

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SUPPLEMENTARY MATERIALS

Supplementary - Tables - Li et al., 2024

Download: https://ija.scholasticahq.com/article/120180-comparative-analysis-of-the-structural-and-compositional-change-of-spotted-sea-bass-_lateolabrax-maculatus_-gut-microflora-following-_aeromonas-vero/attachment/232685.pdf