

Original Research Articles

Effects of *Astragalus* polysaccharides on erysipelas and intestinal microbiota of *Whitmania pigra*

Kai Ma¹, Guiyan Shao¹, Hongwei Ao², Bo Huang¹, Mengxue Li¹, Chao Feng¹, Chenbo Sun¹, Hongyan Tian¹, Wenping Yang¹, Kui Zhang², Boxing Cheng^{2a}, Fei Liu^{1b}¹ School of Marine and Bioengineering, Yancheng Institute of Technology, Yancheng 224051, China, ² School of Biological Sciences, Guizhou Education University, Guiyang 550018, ChinaKeywords: erysipelas, APS, microbiota, *Whitmania pigra*<https://doi.org/10.46989/001c.163024>

Israeli Journal of Aquaculture - Bamidgah

Vol. 78, Issue 2, 2026

Whitmania pigra (Whitman leech) is an aquatic annelid with extensive pharmacological activities. Erysipelas has severely affected the development of its artificial aquaculture industry. *Astragalus* polysaccharide (APS), as an immunopotentiator, is often used in aquaculture to enhance disease resistance of aquatic animals. However, the effect of APS on the intestinal microbiota of *W. pigra* remains unclear. In this study, 16S rDNA amplicon sequencing was used to explore the effects of APS on the prevention and control of “erysipelas” and the intestinal microbial community of *W. pigra*. The results showed that after injection with APS, the abundances of Bacteroidota and Firmicutes in the leech intestinal tract increased, while those of Proteobacteria decreased. At the genus level, the relative abundance of *Bacteroides* increased significantly. The results indicate that APS can optimize the intestinal microbial community structure of *W. pigra*, correct the imbalance in flora composition, thereby enhancing the body’s immunity and effectively preventing and controlling erysipelas, providing a theoretical reference for green prevention and control of disease in the aquaculture of this species.

1. INTRODUCTION

Whitmania pigra is listed in the Chinese Pharmacopoeia (2025 Edition) as one of the original animal sources of the medicinal leech. It plays an irreplaceable role in the clinical treatment of cardiovascular diseases such as myocardial infarction and cerebral thrombosis. As the primary species of artificially farmed leech in China,^{1,2} With the continuous surge in market demand for medicinal leech materials, the wild resources of *W. pigra* have been declining annually due to overfishing and habitat destruction, and large-scale artificial aquaculture has become the core approach to fill the resource gap. However, with higher cultivation density and greater complexity of the aquaculture environment, disease issues in *W. pigra* have become increasingly prominent. Among these, erysipelas caused by *Aeromonas allosaccharophila* is characterized by an acute onset,³ rapid transmission and high mortality. This has become a critical bottleneck restricting the healthy development of the artificial *W. pigra* aquaculture industry, inflicting severe economic losses on farmers and indirectly compromising the stable supply of medicinal leech resources.

Intestinal probiotics can not only promote the digestion and absorption of nutrients and energy substances in aquatic animals but also enhance their immune functions.^{4,5} Together, the intestines of aquatic animals and their symbiotic flora form a biochemical barrier against pathogenic attacks and environmental stresses, playing a crucial role in the host’s immune function. An imbalance in the intestinal microbiota can trigger various diseases.⁶ In *Tachysurus fulvidraco* suffering from intestinal septicemia, the intestinal microbiota diversity decreased significantly, with a marked enrichment of the pathogenic bacterium *Edwardsiella ictaluri* and a reduction in the abundance of probiotics such as *Cetobacterium*.⁷ Similar trends of intestinal microbial changes have also been observed in diseased aquatic animals, including *Penaeus monodon*,⁸ *Paralichthys olivaceus*,⁹ and *Cyprinus carpio*.¹⁰ Unlike vertebrate fish that possess both innate and adaptive immunity, the invertebrate annelid *W. pigra* relies entirely on the innate immune system.^{11,12} Given the absence of adaptive immunity, all immunomodulators applied to *W. pigra* exert their effects by boosting components of innate immunity, rather than via lymphocyte-dependent memory responses.

a Corresponding author. Boxing Cheng, E-mail: cbx@gznc.edu.cnb Corresponding author. Fei Liu, E-mail: liufei@ycit.edu.cn

Astragalus polysaccharide, one of the main extracts of *Astragalus membranaceus*, has significant biological activities, including immunomodulation, anti-inflammatory, and antioxidative effects.¹³ As a feed additive, APS can promote intestinal development, regulate the balance of the intestinal microbiota, improve the immune performance of the organism, and further inhibit the growth of pathogenic microorganisms.¹⁴ APS has been shown to improve the growth performance of *Macrobrachium rosenbergii* and enhance its innate immune responses as well as resistance against *Vibrio anguillarum*.¹⁵ APS significantly reduces the incidence of *Vibrio alginolyticus* infection in *Larimichthys crocea* cultures.¹⁶ APS improves growth performance by enhancing antioxidant function, increasing nonspecific immunity, regulating the Toll/NF- κ B pathway and the proPO system, and optimizing the intestinal microbiota of *Eriocheir sinensis*.¹⁷ APS has also been applied to regulate the intestinal microbiota and prevent pathogenic infections in the artificial cultivation of various aquatic animals, including *Epinephelus fuscoguttatus*,¹⁸ *Oreochromis niloticus*,¹⁹ and *Micropterus salmoides*.²⁰ Intramuscular injection was adopted in this study to ensure accurate dose administration and synchronized immune stimulation, thereby eliminating confounding interference from variations in food intake during *W. pigra* feeding behavior. This approach conforms to established protocols for parenteral administration in aquatic animals, and the injection route is widely utilized in applied fundamental research.^{21,22}

In the artificial cultivation of *W. pigra*, frequent disease outbreaks often result in large-scale mortality, severely hindering the sustainable development of this industry. Among these diseases, “erysipelas”, which predominantly occurs during the high-temperature period from mid-to-late August every year, is particularly prominent. Infected individuals exhibit symptoms, including muscle rigidity in the clitellar region, body-surface erythema and swelling, and coelomic effusion, and are associated with high mortality rates, thus becoming a key limiting factor in aquaculture production. Previous studies have demonstrated that APS can promote the growth of *W. pigra* and enhance its immunity and tolerance to heat stress²³; however, systematic research on the specific preventive and therapeutic effects of this polysaccharide against erysipelas, as well as on whether it affects host immune mechanisms by regulating the intestinal microbiota, is still lacking. In this study, 16S rDNA amplicon sequencing was used to systematically analyze the effects of *Astragalus* polysaccharide on the prevention and control of “erysipelas” and the intestinal microbial community of *W. pigra*, aiming to reveal its potential mechanism of action and provide new theoretical basis and practical guidance for the prevention and treatment of erysipelas.

2. MATERIALS AND METHODS

2.1. REAGENTS AND PREPARATION

Astragalus polysaccharide with a purity of $\geq 90.0\%$ and a molecular weight of 254.69 Da was purchased from Beijing

Solarbio Science & Technology Co., Ltd. It is composed of hexuronic acid, glucose, fructose, rhamnose, arabinose, galacturonic acid, and glucuronic acid. The contents of water, ash, and lead were all less than 10%. Sterile distilled water was used as the solvent for APS injection at a concentration of $3.0 \text{ mg}\cdot\text{mL}^{-1}$.

2.2. EXPERIMENTAL ANIMALS AND EXPERIMENTAL DESIGN

The *W. pigra* used in this study were obtained from an ecological leech farm in Xinghua County, Jiangsu Province, China. A total of 120 individuals with a body weight of $2.5 \pm 0.5 \text{ g}$ were used. Species identification was performed according to the morphological description in Fauna Sinica: Hirudinea, and was further confirmed by COI gene sequencing.²⁴ All individuals were verified as *W. pigra*. Healthy leeches were randomly assigned to 6 aquariums with dimensions of 30 cm (L) $\times$ 30 cm (W) $\times$ 30 cm (H), with 20 leeches per aquarium. The leeches were divided into a treatment group and a control group, with 3 biological replicates per group.

In the 6 aquariums designated for sequencing analysis, each leech in the treatment group was intramuscularly injected with 0.4 mL of APS solution at a dose of 0.02 mg/g body weight,²¹ whereas each leech in the control group was intramuscularly injected with 0.4 mL of sterile distilled water as a placebo. *Aeromonas allosaccharophila*, a pathogenic bacterium previously isolated and identified in our laboratory, was activated and cultured. The bacterial suspension was serially diluted with sterile water to a final concentration of $1.0 \times 10^8 \text{ CFU/mL}$. *W. pigra* in both control and treatment groups were subjected to an immersion challenge using a bacterial suspension of $1.0 \times 10^8 \text{ CFU/mL}$. During the experiment, the health status of the leeches was observed and recorded daily. Leeches suffering from erysipelas presented with abdominal congestion and hemorrhage, decreased body elasticity, sucker swelling, and reduced adhesion ability. As the disease progresses, severe rotting of the head and tail, coelomic effusion, and clitellum redness and swelling occur.³ In the treatment group, diseased and dead individuals were labeled Ainfl, and surviving individuals were labeled AnorI; in the control group, diseased and dead individuals were labeled infl, and surviving individuals were labeled norI. Moreover, aquaculture water samples were collected and labeled InfW. Healthy leeches without APS injection and bacterial challenge were used as blank controls and labeled CK. The number of dead leeches in each of the 6 groups was recorded, and the survival rate (SR) was calculated via the following formula: $\text{SR} (\%) = 100 \times (\text{Total number} - \text{Number of deaths}) / \text{Total number}$.

During the culture period, the leeches were fed river snails twice a day. Continuous aeration was maintained, and the water was changed daily. The water temperature was maintained at $26.86 \pm 1.65^\circ\text{C}$, measured at 8:00 and 17:00 each day. Water quality was monitored every 3 days to maintain the following conditions: pH 6.50–7.50, hydrogen sulfide (H_2S) concentration $< 0.01 \text{ mg/L}$, ammonia (NH_3) concentration $< 0.04 \text{ mg/L}$, and dissolved oxygen (DO) concentration $> 2.0 \text{ mg/L}$.

2.3. SAMPLE COLLECTION

Intestinal samples were collected at the end of the experiment (Day 6). New gloves were worn for each sample collection to avoid cross-contamination. Leeches were rinsed with sterile autoclaved distilled water, anesthetized on ice, and dissected using sterile instruments. Intestinal tissues and their contents were rapidly collected, placed separately into individual 5 mL sterile cryogenic vials, and immediately frozen in liquid nitrogen. All samples were stored at -80°C in an ultra-low temperature freezer until subsequent analysis.

2.4. INTESTINAL MICROBIOTA ANALYSIS

2.4.1. GENOMIC DNA EXTRACTION, PCR AMPLIFICATION, AND SEQUENCING

Genomic DNA was extracted from intestinal samples via the SDS method. The quality and concentration of the extracted DNA were detected via 1% agarose gel electrophoresis and a nucleic acid and protein analyzer (Implen GmbH, Munich, Germany), and the DNA was diluted to $1\text{ ng}\cdot\mu\text{L}^{-1}$ with sterile water. A total of 30 qualified genomic DNA samples, distributed across 5 groups, were sent to Beijing Novogene Co., Ltd. for PCR amplification. The V3–V4 hypervariable regions of the 16S rDNA were amplified via bacterial universal primer pairs with barcodes (341F: CC-TAYGGGRBGCASCAG; 806R: GGACTACNNGGGTATCTAAT). After the PCR products were detected by 2% agarose gel electrophoresis, equal volumes were pooled and purified using the Qiagen Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). Library construction was performed with the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs Inc., Ipswich, MA, USA), and the library quality was evaluated via a Qubit® 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer 2100 System (Agilent Technologies, Inc., Santa Clara, CA, USA). Finally, paired-end sequencing was conducted on the Illumina NovaSeq 6000 platform (Illumina, Inc., San Diego, CA, USA).

2.4.2. BIOINFORMATICS ANALYSIS

Paired-end sequencing reads were assigned to their corresponding samples using barcodes, and the barcodes and primer sequences were trimmed. The reads for each sample were assembled using FLASH (V1.2.11) to generate raw tags.²⁵ Quality control was performed with fastp (V0.20.0) software to obtain clean tags. Clean tags were aligned against the Silva 138.1 database via Vsearch (V2.15.0), and chimeras were removed to yield effective tags.²⁶ Denoising and filtering were performed using the DADA2 module in QIIME2 (Version QIIME2-202006) to obtain the final amplicon sequence variants (ASVs) and feature tables.^{27,28} Finally, the ASVs were aligned against the reference database via QIIME2 software to obtain taxonomic information for each ASV, followed by multiple sequence alignment.

Alpha and beta diversity analyses of the intestinal microbial community were performed using QIIME2. Alpha

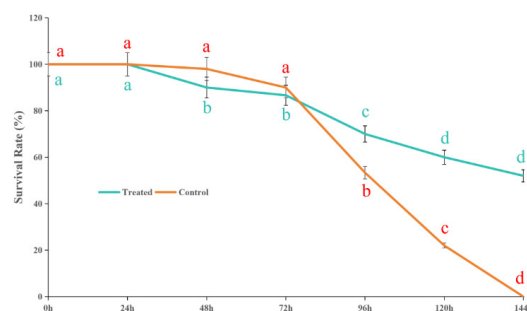


Figure 1. Survival curves of *W. pigra* in the APS injection group (0.02 mg/g body weight) and the control group (without APS injection).

According to two-way ANOVA, different lowercase letters at each sampling point indicate significant differences at the 0.05 probability level among different periods.

diversity measures the richness and evenness of microbial species within a sample.²⁹ The Shannon and Simpson indices were selected to reflect community diversity, whereas the Observed_otus and Chao1 indices were used to evaluate species richness.³⁰ For beta diversity analysis, the non-metric multidimensional scaling (NMDS) method was adopted.³¹ Ordination plots were constructed using weighted and unweighted UniFrac distances to reveal differences in microbial community structure and their multidimensional distributions among samples.^{32–34}

Linear discriminant analysis effect size (LEfSe) analysis was performed via LEfSe software (version 1.0),³⁵ with the LDA score threshold set to 4 by default. The length of the bar plot represents the effect size of the differential species (i.e., LDA score). Functional prediction was performed using Tax4Fun software based on 16S rDNA sequencing data.³⁶

2.5. STATISTICAL ANALYSIS

The relevant charts were plotted using Origin 2021. The intestinal microbiota data were analyzed via the Novogene Magic Cloud Platform (<https://magic.novogene.com/customer/main#/homeNew/3adfe99ed18ed30596999d5ce47b939a>).

3. RESULTS

3.1. EFFECTS OF ASTRAGALUS POLYSACCHARIDE ON THE SURVIVAL RATE OF *WHITMANIA PIGRA*

During the experiment, individual deaths began to occur on the second day. By the sixth day, all individuals in the control group had died, while the survival rate of the *W. pigra* group injected with APS remained at 50%. The changes in survival rates across the experimental groups are shown in Fig. 1.

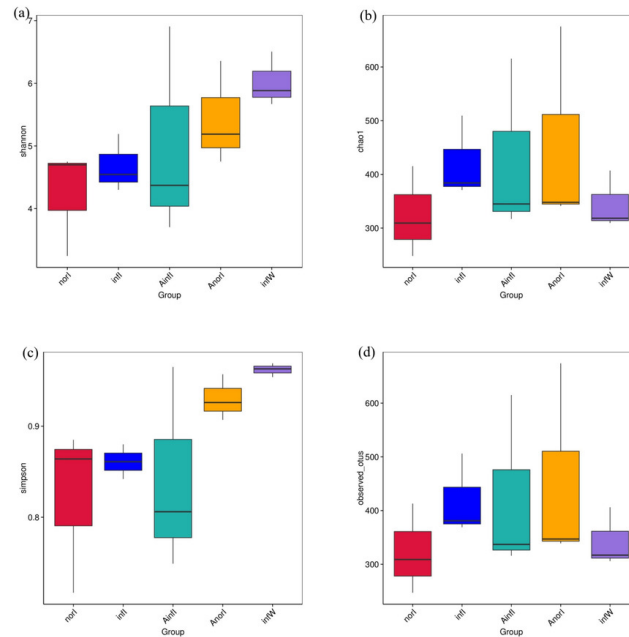


Figure 2. Alpha diversity of intestinal microbiota in dead and surviving *W. pigra* before and after APS injection.

(a) Shannon index, (b) Chao1 index, (c) Simpson index, and (d) Observed_otus index.

3.2. EFFECTS OF APS ON THE ALPHA AND BETA DIVERSITY OF THE INTESTINAL MICROBIOTA IN *W. PIGRA*

Detailed information on the diversity and richness indices of the intestinal microbiota in *W. pigra* is presented in Fig. 2. Compared with those in the control groups (infl and norI), the Shannon index (Fig. 2a), Chao1 index (Fig. 2b), Simpson index (Fig. 2c), and Observed_otus index (Fig. 2d), as well as the richness indices, were significantly greater in the treatment groups (Ainfl and AnorI).

Beta diversity differences in the intestinal microbiota between the control and treatment groups of *W. pigra* were evaluated via nonmetric multidimensional scaling (NMDS) based on weighted UniFrac (Fig. 3a) and unweighted UniFrac (Fig. 3b) distances. The stress values of the NMDS plots were 0.089 and 0.074, respectively, indicating a good fit of the ordination results. The analysis revealed significant differences in microbial community structure between the two groups, with distinct clustering and separation in the plots.

3.3. EFFECTS OF APS ON THE INTESTINAL MICROBIOTA COMPOSITION OF *W. PIGRA*

At the phylum level, the dominant phyla in the norI group were Proteobacteria, Firmicutes, and Bacteroidota. The dominant phyla in the infl group were also Proteobacteria, Firmicutes, and Bacteroidota. In the Ainfl group, the dominant phyla were Bacteroidota, Firmicutes, and Proteobacteria. The dominant phyla in the AnorI group were Firmicutes, Bacteroidota, and Proteobacteria (Fig. 4a).

At the genus level, the dominant genera in the norI group were *Aminobacter*, *Phreatobacter*, and *Delftia*; those

in the infl group were *Aminobacter*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Anaerorhabdus_furcosa_group*; those in the Ainfl group were *Bacteroides*, *Ralstonia*, and *Phreatobacter*; and those in the AnorI group were *Anaerorhabdus_furcosa_group*, *Bacteroides*, and *Aminobacter*, respectively (Fig. 4b).

3.4. DIFFERENTIAL ABUNDANCE ANALYSIS OF THE INTESTINAL MICROBIOTA COMPOSITION IN *W. PIGRA*

LEfSe analysis revealed that, at the genus level, *Aminobacter* and *Nubsella* were significantly enriched in the AnorI group compared with the other subgroups in the APS injection group (Fig. 5a), whereas *Mucispiillum* was significantly enriched in the Ainfl group. In the control group, comparisons within subgroups revealed that the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Anaerorhabdus_furcosa_group* were significantly enriched in the infl group, whereas *Aminobacter*, *Phreatobacter*, *Acetobacterium*, *Nubsella* and *Methanobrevibacter* were significantly enriched in the norI group (Fig. 5b).

Pairwise comparisons among all four groups revealed that, at the genus level, *Aminobacter*, *Acetobacterium*, *Nubsella* and *Methanobrevibacter* in the norI group, as well as *Mucispiillum* in the Ainfl group, achieved relatively high LDA scores (Fig. 5c). Moreover, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* in the infl group also had relatively high LDA scores (Fig. 5d).

3.5. EFFECTS OF APS ON THE INTESTINAL MICROBIOTA OF *W. PIGRA*

On the basis of the Tax4Fun tool and KEGG database, functional prediction and annotation of the metagenome represented by ASVs were performed. The functional pathways

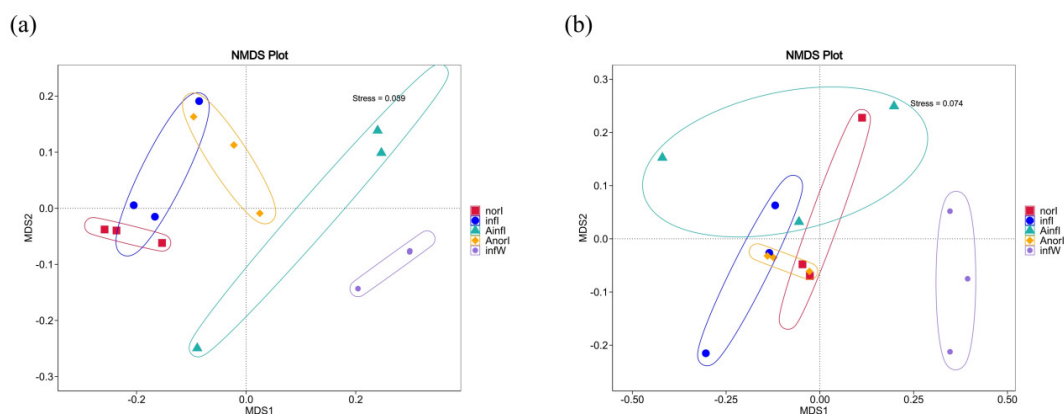


Figure 3. Beta diversity of intestinal microbiota in dead and surviving *W. pigra* before and after APS injection based on NMDS analysis.

(a) NMDS plot based on Weighted_unifrac distance, (b) NMDS plot based on Unweighted_unifrac distance.

were classified into three levels (Level 1, Level 2, and Level 3) according to the KEGG hierarchical system. Among the intestinal samples from both the control and *W. pigra* treatment groups, metabolism was the most dominant level 1 functional category, followed by genetic information processing, environmental information processing, and cellular processes. Compared with the control group, the relative abundances of functional pathways related to metabolism and organismal systems in the treatment group were significantly higher, whereas those related to environmental information processing were significantly lower (Fig. 6a).

In the KEGG level 2 metabolic pathway classification, differences between intestinal samples before and after treatment were mainly reflected in pathways related to carbohydrate metabolism, membrane transport, and amino acid metabolism, whereas other gene functions were mainly involved in energy metabolism, nucleotide metabolism, and other pathways. The relative abundances of the above functional pathways differed significantly across all samples, indicating that APS treatment and disease progression significantly affected the functional composition of the intestinal microbiota in *W. pigra* (Fig. 6b).

In the classification of KEGG level 3 metabolic pathways of intestinal bacteria in the treatment group, the proportions of sequences related to carbon fixation pathways in prokaryotes; transport, amino sugar and nucleotide sugar metabolism; and galactose metabolism were significantly increased. In contrast, the abundances of pathways related to the secretion system and two-component system were significantly greater in the control group (Fig. 6c).

4. DISCUSSION

Astragalus polysaccharide is a feed additive and immunoadjuvant widely used in aquaculture. Studies have demonstrated that it can not only enhance the immune efficacy of inactivated vaccines but also increase the activities of various immune-related enzymes and the expression levels of immune genes.³⁷ When combined with inactivated vaccines, it can alleviate splenic tissue damage in rainbow trout (*Oncorhynchus mykiss*) infected with infectious hematopoietic necrosis virus (IHNV).³⁸ A diverse microbial ecosystem is crucial for resisting pathogenic invasion.³⁹ By improving the intestinal microbiota structure of aquatic animals, APS can increase the proportion of probiotics and the expression of intestinal immune factors, thereby enhancing disease resistance and survival rates. These effects have been verified in various aquatic species, including *Pelteobagrus fulvidraco*,⁴⁰ *Eriocheir sinensis* and *Acipenser dabryanus*.^{17,41} In this study, we also found that APS treatment significantly increased the richness and diversity of the intestinal microbiota in *W. pigra* (Fig. 2). Furthermore, β -diversity analysis based on nonmetric multidimensional scaling (NMDS) revealed significant differences in community structure between the treatment group and the control group (Fig. 3). The intestinal microbiota of both the experimental group and the control group was predominantly composed of the phyla Bacteroidota, Proteobacteria, and Firmicutes, which is consistent with previous research.⁴² APS treatment altered the relative abundances of the dominant phyla: Bacteroidota and Firmicutes were the dominant phyla in the APS-treated group, whereas Proteobacteria was the most abundant phylum in the control group. Proteobacteria include many pathogenic species, and their

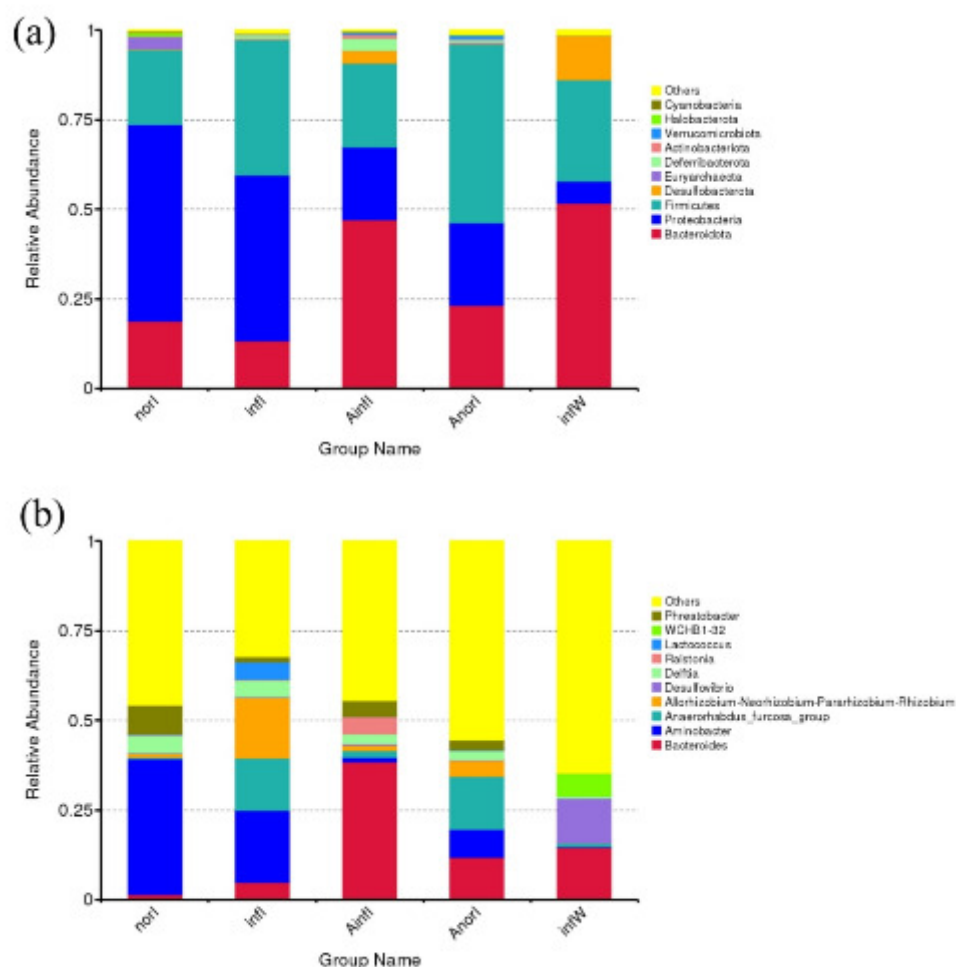


Figure 4. Analysis of intestinal microbiota composition in dead and surviving *W. pigra* before and after APS injection.

(a) Top 10 phyla with the highest relative abundance in each group, (b) Top 10 genera with the highest relative abundance in each group.

high abundance is often associated with the development of intestinal diseases in the host. The decreased relative abundance of Proteobacteria and increased relative abundances of Bacteroidota and Firmicutes in the APS-treated group suggested that APS can reduce the colonization of pathogenic bacteria in the intestine, promote the proliferation of beneficial bacteria, and thus facilitate the recovery of intestinal health.

Compared with the control group, the treatment group showed a decrease in Proteobacteria relative abundance, whereas Bacteroidota relative abundance increased. Moreover, the abundance of *Firmicutes* in the AnorI group was significantly greater than that in the norI group. These results are consistent with the findings of previous studies on *Ctenopharyngodon idellus*,⁴³ zebrafish, and *Plectropomus leopardus* supplemented with APS.^{44,45} At the genus level, the relative abundance of *Bacteroides* in the treatment group was significantly greater than that in the control group. This result aligns with the outcomes of studies involving *Ctenopharyngodon idellus* fed a Sudan grass diet and *Pelophylax nigromaculatus* fed a phytogetic diet.^{46,47} *Bacteroides* can decompose complex carbohydrates and pro-

duce SCFAs, which regulate the intestinal immune system,⁴⁸ promote immune maturation, reduce inflammation,⁴⁹ and inhibit pathogenic microorganisms, thereby improving intestinal health.⁵⁰

The primary metabolic products of Bacteroidota are acetate and propionate, while Firmicutes mainly produce butyrate.⁵¹ The *Bacteroides* and *Anaerorhabdus_furcosa_group* mentioned in this study belong to Bacteroidota and Firmicutes, respectively, and both are capable of producing SCFAs. The significant increase in the abundances of these two bacterial taxa can promote immune system maturation, inhibit proinflammatory pathways, and reduce the risk of intestinal inflammation. In addition, the abundances of *Ralstonia*, *Mucispirillum* and *Aminobacter*, which are members of Proteobacteria, distinctly changed in moribundly infected individuals: the abundances of the first two genera increased significantly, whereas that of *Aminobacter* decreased. Abnormal proliferation of Proteobacteria is widely recognized as a hallmark of intestinal dysbiosis.⁵² At present, the ecological functions of *Aminobacter* in invertebrate hosts remain unclear, and the underlying mechanisms for its downregulation during the disease course

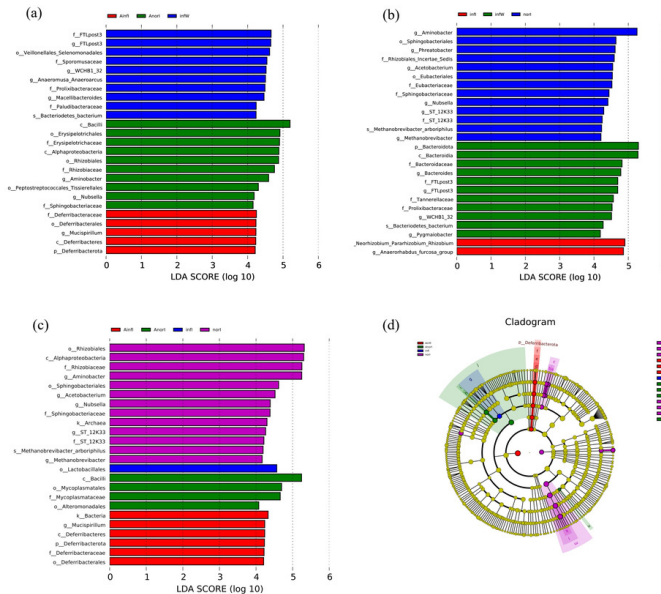


Figure 5. Analysis of species diversity and biomarker identification of intestinal microbiota in *W. pigra* between the treatment group and the control group.

(a–d) Analysis of microbial species differences among groups: (a) Ainfl vs Anorl, (b) infl vs norl, (c) Integrated LEfSe analysis of treatment group and control group (LDA > 4), (d) Phylogenetic tree of microbial biomarkers.

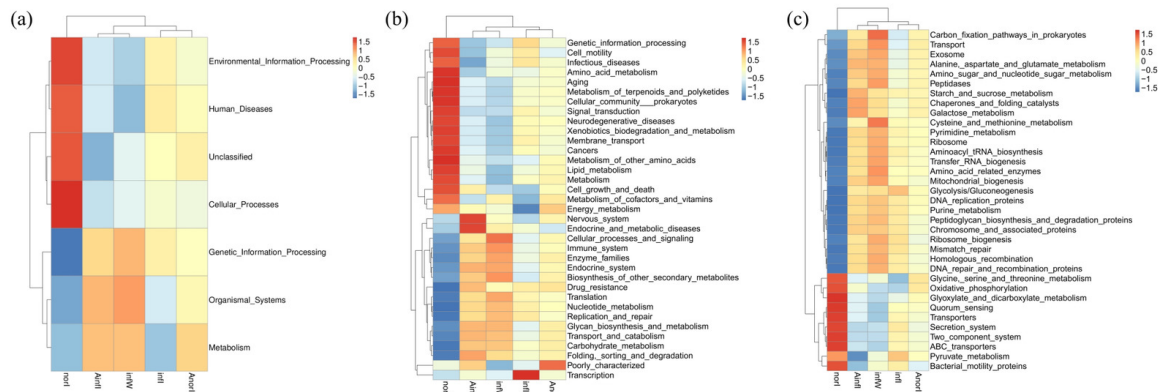


Figure 6. Tax4Fun clustering heatmap of *W. pigra* in treatment and control groups.

(a) Level 1 functional hierarchy showing all metabolic pathways, (b) Level 2 functional hierarchy including all pathways, (c) Level 3 functional hierarchy presenting all significant functional features related to xenobiotic degradation.

warrant further investigation. Bacteroidetes, Firmicutes and Proteobacteria play pivotal roles in host metabolism, and an imbalance in the ratio of these three phyla may induce metabolic disorders.⁵³ Functional prediction analysis revealed that the intergroup differences were mainly concentrated in metabolism-related pathways, suggesting a close association between APS and the metabolic functions of the intestinal microbiota. These results demonstrate that APS can improve intestinal health and enhance host disease resistance in *W. pigra* by increasing the richness and diversity of the intestinal microbiota; regulating the relative abundances of dominant phyla, including Bacteroidota, Firmicutes and Proteobacteria; enriching beneficial bacter-

ial genera that produce SCFAs; and inhibiting the proliferation of harmful bacteria.

Notably, intestinal microbiota analysis in this study was only performed at the experimental endpoint on day 6 post-challenge with *Aeromonas allosaccharophila*. At this time point, all *W. pigra* in the control group had succumbed to erysipelas, whilst the survival rate of individuals in the APS-treated group reached 50%. Although this sampling time point can highlight the greatest discrepancies in microbial communities between surviving and diseased leeches, sampling at a single time point makes it difficult to infer the temporal causal relationship between shifts in microbial composition and disease progression. It remains

unclear whether the observed microbiota alterations occur before, concurrently with, or after the onset of erysipelas. Further research is recommended to adopt longitudinal gradient sampling on days 0, 2, 4, and 6 post-challenge to elucidate the dynamic succession of intestinal microbiota and clarify the intrinsic functional mechanisms linking APS intervention, intestinal microbial homeostasis, and disease resistance in *W. pigra*.

This study confirmed that APS injection effectively improved the survival rate of *W. pigra* infected with *Aeromonas allosaccharophila*. Comparative analysis of intestinal microbiota in leeches before and after infection revealed that astragalus polysaccharides optimized the intestinal microbial structure, increased the abundance of beneficial bacteria, and inhibited pathogenic bacteria. As a natural, safe, and effective feed additive and immunopotentiator, APS has great potential for preventing and controlling diseases in *W. pigra* aquaculture.

5. CONCLUSIONS

This study deepens understanding of the mechanism by which *Astragalus* polysaccharide regulates the intestinal microbiome of *W. pigra* and highlights the importance of research on the interactions between the active ingredients of traditional Chinese medicine and the microbiome. Although we have identified indicative microbial biomarkers, the specific molecular mechanism by which *Astragalus* polysaccharides affect host immunity and growth performance via the intestinal microbiota remains unclear. Furthermore, excluding the healthy blank control group (CK) from microbiota sequencing analyses impedes the reliable definition of the baseline microbiome of *W. pigra*, a notable limitation that ought to be addressed in future research. Future research should focus on the regulatory network among *Astragalus* polysaccharide, microbiota, and host, so as to improve the systematic understanding of the mechanism by which traditional Chinese medicine ingredients regulate the health of aquatic animals. In addition, although *Astragalus* polysaccharide shows application prospects as a potential prebiotic, dose-response experiments and long-term intervention studies are still needed to evaluate its optimal efficacy in regulating intestinal flora structure and enhancing disease resistance. Given the urgent need to reduce antibiotic use in aquatic animal farming, an integrated health management strategy combining traditional Chinese medicine with modern microbiome

technologies is of great significance for the sustainable development of aquatic economic species such as *W. pigra*.

ACKNOWLEDGEMENT

This research was financially supported by the National Natural Science Foundation of China (NSFC, grant no. 82474041 and 82073968), China; The Doctoral Program of the Science Research Foundation of Guizhou Education University (2024BS006 and 2024BS011), China; Reward and subsidy fund project of Guizhou Education University, Ministry of science and technology of the people's Republic of China and National Natural Science Foundation of China (2023GZJB004), China. Guizhou Forestry Administration Scientific Research Project (QLKH [2023] 11 and QLKH [2025] 11), China; Guizhou Science and Technology Support Plan Project ([2023]188), China.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Kai Ma. *Methodology:* Kai Ma. *Data curation:* Fei Liu. *Formal Analysis:* Bo Huang, Mengxue Li, Chao Feng, Wenping Yang. *Investigation:* Guiyan Shao, Hongwei Ao. *Software:* Kai Ma. *Writing – original draft:* Kai Ma. *Resources:* Fei Liu. *Validation:* Chenbo Sun, Hong-Yan Tian. *Writing – review & editing:* Boxing Cheng. *Project administration:* Boxing Cheng. *Supervision:* Kui Zhang.

ETHICS APPROVAL

Ethical approval is not required for the paper.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Submitted: April 28, 2026 CDT. Accepted: May 30, 2026 CDT.

Published: June 25, 2026 CDT.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CCBY-4.0). View this license's legal deed at <http://creativecommons.org/licenses/by/4.0> and legal code at <http://creativecommons.org/licenses/by/4.0/legalcode> for more information.

REFERENCES

1. Liu J, Wang L, Duan W, Zhang Y, Jiang Q. Traditional uses, chemical constituents, pharmacological activities and toxicology of Chinese medicinal leech (Shuizhi): A comprehensive review. *Journal of Ethnopharmacology*. 2026;355:120634. doi:[10.1016/j.jep.2025.120634](https://doi.org/10.1016/j.jep.2025.120634)
2. Ye T, Zhao F, Xiao M, et al. Comparative Study of Lefaxin Family in Two Asian Leeches: *Hirudinaria manillensis* and *Whitmania pigra*. *Biology*. 2025;14:918. doi:[10.3390/biology14080918](https://doi.org/10.3390/biology14080918)
3. Shao G, Wang S, Jiang X, et al. Isolation, identification and characterization of *Aeromonas allosaccharophila* from infected farmed leech (*Whitmania pigra*) in Jiangsu, China. *Aquaculture*. 2023;576:739867. doi:[10.1016/j.aquaculture.2023.739867](https://doi.org/10.1016/j.aquaculture.2023.739867)
4. Ringo E, Harikrishnan R, Soltani M, Ghosh K. The effect of gut microbiota and probiotics on metabolism in fish and shrimp. *Animals*. 2022;12:3016. doi:[10.3390/ani12213016](https://doi.org/10.3390/ani12213016)
5. Zhang Z, Yang Q, Liu H, et al. Potential functions of the gut microbiome and modulation strategies for improving aquatic animal growth. *Reviews in Aquaculture*. 2025;17:e12959. doi:[10.1111/raq.12959](https://doi.org/10.1111/raq.12959)
6. MedinaFélix D, GaribayValdez E, VargasAlbores F, MartínezPorchas M. Fish disease and intestinal microbiota: A close and indivisible relationship. *Reviews in Aquaculture*. 2023;15:820-839. doi:[10.1111/raq.12762](https://doi.org/10.1111/raq.12762)
7. Yang J, Lin Y, Wei Z, et al. *Edwardsiella ictaluri* almost completely occupies the gut microbiota of fish suffering from enteric septicemia of catfish (Esc). *Fishes*. 2023;8:30. doi:[10.3390/fishes8010030](https://doi.org/10.3390/fishes8010030)
8. Angthong P, Uengwetwanit T, Uawisetwathana U, et al. Investigating host-gut microbial relationship in *Penaeus monodon* upon exposure to *Vibrio harveyi*. *Aquaculture*. 2023;567:739252. doi:[10.1016/j.aquaculture.2023.739252](https://doi.org/10.1016/j.aquaculture.2023.739252)
9. Kim D, Lee S, Lee JM, Lee E, Jang WJ. Changes in the gut microbiota composition of juvenile olive flounder (*Paralichthys olivaceus*) caused by pathogenic bacterial Infection. *Fishes*. 2023;8:294. doi:[10.3390/fishes8060294](https://doi.org/10.3390/fishes8060294)
10. Chang S, Wang J, Dong C, Jiang Y. Intestinal microbiota signatures of common carp (*Cyprinus carpio*) after the infection of *Aeromonas hydrophila*. *Aquaculture Reports*. 2023;30:101585. doi:[10.1016/j.aqrep.2023.101585](https://doi.org/10.1016/j.aqrep.2023.101585)
11. Madhusudhan N, Nair P, Kale RD. Isolation and culturing of earthworm (*Eudrilus eugeniae*) coelomocytes. *Dynamic Soil, Dynamic Plant*. 2009;3:111-114.
12. Prochazkova P, Roubalova R, Dvorak J, Navarro Pacheco NI, Bilej M. Pattern recognition receptors in annelids. *Developmental & Comparative Immunology*. 2020;102:103493. doi:[10.1016/j.dci.2019.103493](https://doi.org/10.1016/j.dci.2019.103493)
13. Liang H, Tao S, Wang Y, et al. *Astragalus* polysaccharide: implication for intestinal barrier, anti-inflammation, and animal production. *FRONTIERS IN NUTRITION*. 2024;11:1364739. doi:[10.3389/fnut.2024.1364739](https://doi.org/10.3389/fnut.2024.1364739)
14. Li W, Zeng J, Zheng N, et al. *Astragalus* polysaccharide slows the dissemination of antibiotic resistance genes and reduces the prevalence of opportunistic pathogens in the fish gut. *Journal of Environmental Management*. 2025;389:126058. doi:[10.1016/j.jenvman.2025.126058](https://doi.org/10.1016/j.jenvman.2025.126058)
15. Sun S, Bian C, Zhou N, Shen Z, Yu M. Dietary *Astragalus* polysaccharides improve the growth and innate immune response of giant freshwater prawn *Macrobrachium rosenbergii*: Insights from the brain-gut axis. *International Journal of Biological Macromolecules*. 2023;243:125158. doi:[10.1016/j.ijbiomac.2023.125158](https://doi.org/10.1016/j.ijbiomac.2023.125158)
16. Zhang W, Wang Y, Cheng A, et al. Protective effects of dietary *Astragalus* polysaccharides on large yellow croaker (*Larimichthys crocea*) against *Vibrio alginolyticus* infection. *Aquaculture*. 2024;581:740398. doi:[10.1016/j.aquaculture.2023.740398](https://doi.org/10.1016/j.aquaculture.2023.740398)
17. Shi X, Wu C, Ma H, et al. Exploring the effects of astragalus polysaccharide food supplementation on growth performance, immunity, antioxidant capacity, and intestinal microflora in Chinese mitten crabs. *Aquaculture Reports*. 2024;34:101908. doi:[10.1016/j.aqrep.2023.101908](https://doi.org/10.1016/j.aqrep.2023.101908)
18. Wei G, Cai S, Wu Y, Ma S, Huang Y. Immune effect of *Vibrio harveyi* formalin-killed cells vaccine combined with chitosan oligosaccharide and astragalus polysaccharides in ♀*Epinephelus fuscoguttatus* × ♂*Epinephelus lanceolatus*. *Fish & Shellfish Immunology*. 2020;98:186-192. doi:[10.1016/j.fsi.2020.01.015](https://doi.org/10.1016/j.fsi.2020.01.015)

19. Zahran E, Risha E, AbdelHamid F, Mahgoub HA, Ibrahim T. Effects of dietary *Astragalus* polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*. 2014;38:149-157. doi:[10.1016/j.fsi.2014.03.002](https://doi.org/10.1016/j.fsi.2014.03.002)
20. Lin S, Jiang Y, Chen Y, Luo L, Doolgindachbaporn S, Yuangsoi B. Effects of *Astragalus* polysaccharides (APS) and chitoooligosaccharides (COS) on growth, immune response and disease resistance of juvenile largemouth bass, *Micropterus salmoides*. *Fish & Shellfish Immunology*. 2017;70:40-47. doi:[10.1016/j.fsi.2017.08.035](https://doi.org/10.1016/j.fsi.2017.08.035)
21. Lin G, Da F, Wan X, et al. Immune-enhancing effects of *Astragalus* polysaccharides and *Ganoderma lucidum* polysaccharides on *Vibrio harveyi* flgJ DNA vaccine in grouper. *Journal of Fish Diseases*. 2023;46:147-156. doi:[10.1111/jfd.13728](https://doi.org/10.1111/jfd.13728)
22. Yuan C, Pan X, Gong Y, et al. Effects of *Astragalus* polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio* L. *International Immunopharmacology*. 2008;8:51-58. doi:[10.1016/j.intimp.2007.10.009](https://doi.org/10.1016/j.intimp.2007.10.009)
23. Shi H, Wu B, Shi G, Zhu Z. The effects of astragalus polysaccharides on the growth, heat stress tolerance and related gene expression of the leech *Whitmania pigra*. *Aquaculture Research*. 2021;52:3247-3255. doi:[10.1111/are.15170](https://doi.org/10.1111/are.15170)
24. Nakanishi H, Yoneyama K, Hara M, Takada A, Saito K. The origin identification method for crude drugs derived from arthropods and annelids using molecular biological techniques. *Journal of Natural Medicines*. 2020;74:275-281. doi:[10.1007/s11418-019-01360-1](https://doi.org/10.1007/s11418-019-01360-1)
25. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 2011;27:2957-2963. doi:[10.1093/bioinformatics/btr507](https://doi.org/10.1093/bioinformatics/btr507)
26. Haas BJ, Gevers D, Earl AM, et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research*. 2011;21:494-504. doi:[10.1101/gr.112730.110](https://doi.org/10.1101/gr.112730.110)
27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*. 2016;13:581-583. doi:[10.1038/nmeth.3869](https://doi.org/10.1038/nmeth.3869)
28. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*. 2019;37:852-857. doi:[10.1038/s41587-019-0209-9](https://doi.org/10.1038/s41587-019-0209-9)
29. Finn DR. A metagenomic alpha-diversity index for microbial functional biodiversity. *FEMS Microbiology Ecology*. 2024;100:fiae019. doi:[10.1093/femsec/fiae019](https://doi.org/10.1093/femsec/fiae019)
30. Li Z, Zhou J, Liang H, et al. Differences in alpha diversity of gut microbiota in neurological diseases. *Frontiers in Neuroscience*. 2022;16:879318. doi:[10.3389/fnins.2022.879318](https://doi.org/10.3389/fnins.2022.879318)
31. Noval Rivas M, Burton OT, Wise P, et al. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *Journal of Allergy and Clinical Immunology*. 2013;131:201-212. doi:[10.1016/j.jaci.2012.10.026](https://doi.org/10.1016/j.jaci.2012.10.026)
32. Lozupone C, Knight R. UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*. 2005;71:8228-8235. doi:[10.1128/AEM.71.12.8228-8235.2005](https://doi.org/10.1128/AEM.71.12.8228-8235.2005)
33. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative β diversity measures lead to different Insights into factors that structure microbial communities. *Applied and Environmental Microbiology*. 2007;73:1576-1585. doi:[10.1128/AEM.01996-06](https://doi.org/10.1128/AEM.01996-06)
34. Investigators I. Beta-diversity distance matrices for microbiome sample size and power calculations – how to obtain good estimates. *Computational and Structural Biotechnology Journal*. 2022;20:2259-2267. doi:[10.1016/j.csbj.2022.04.032](https://doi.org/10.1016/j.csbj.2022.04.032)
35. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biology*. 2011;12:R60. doi:[10.1186/gb-2011-12-6-r60](https://doi.org/10.1186/gb-2011-12-6-r60)
36. Aßhauer KP, Wemheuer B, Daniel R, Meinicke P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics*. 2015;31:2882-2884. doi:[10.1093/bioinformatics/btv287](https://doi.org/10.1093/bioinformatics/btv287)
37. Wan X, Yin Y, Zhou C, et al. Polysaccharides derived from Chinese medicinal herbs: A promising choice of vaccine adjuvants. *Carbohydrate Polymers*. 2022;276:118739. doi:[10.1016/j.carbpol.2021.118739](https://doi.org/10.1016/j.carbpol.2021.118739)

38. Pan Y, Liu Z, Lu J, et al. Astragalus polysaccharide inhibits infectious hematopoietic necrosis virus damage to rainbow trout (*Oncorhynchus mykiss*) spleen by promoting the efficacy of inactivated vaccine. *Fish & Shellfish Immunology*. 2025;159:110180. doi:[10.1016/j.fsi.2025.110180](https://doi.org/10.1016/j.fsi.2025.110180)
39. Kinnunen M, Dechesne A, Proctor C, et al. A conceptual framework for invasion in microbial communities. *The ISME Journal*. 2016;10:2773-2779. doi:[10.1038/ismej.2016.75](https://doi.org/10.1038/ismej.2016.75)
40. Bai C, Huang G, Qi X, et al. Impact of Astragalus polysaccharide, chitosan and xylo-oligosaccharide on intestinal physicochemical properties and gut microbiota disorder of *Pelteobagrus fulvidraco* caused by transport stress. *International Journal of Biological Macromolecules*. 2025;321:146169. doi:[10.1016/j.ijbiomac.2025.146169](https://doi.org/10.1016/j.ijbiomac.2025.146169)
41. Zhang J, Shu D, Cheng X, et al. Effect of plant polysaccharides (*Poria cocos* and *Astragalus* polysaccharides) on immune responses and intestinal microbiota of Dabry's sturgeons. *Bioscience of Microbiota, Food and Health*. 2023;42:243-253. doi:[10.12938/bmfh.2022-089](https://doi.org/10.12938/bmfh.2022-089)
42. Wei W, Shi H, Guo Q, et al. Preliminary study on pathogen of *Whitmania pigra* disease in high temperature environment. *China journal of Chinese materia medica*. 2018;43:3633-3638. doi:[10.19540/j.cnki.cjcmm.20180703.005](https://doi.org/10.19540/j.cnki.cjcmm.20180703.005)
43. Shi F, Lu Z, Yang M, et al. Astragalus polysaccharides mediate the immune response and intestinal microbiota in grass carp (*Ctenopharyngodon idellus*). *Aquaculture*. 2021;534:736205. doi:[10.1016/j.aquaculture.2020.736205](https://doi.org/10.1016/j.aquaculture.2020.736205)
44. Li Y, Ran C, Wei K, et al. The effect of *Astragalus* polysaccharide on growth, gut and liver health, and anti-viral immunity of zebrafish. *Aquaculture*. 2021;540:736677. doi:[10.1016/j.aquaculture.2021.736677](https://doi.org/10.1016/j.aquaculture.2021.736677)
45. Hao X, Lin H, Lin Z, et al. Effect of Dietary *Astragalus* polysaccharides (APS) on the Growth Performance, Antioxidant Responses, Immunological Parameters, and Intestinal Microbiota of Coral Trout (*Plectropomus leopardus*). *Microorganisms*. 2024;12:1980. doi:[10.3390/microorganisms12101980](https://doi.org/10.3390/microorganisms12101980)
46. Hao YT, Wu SG, Xiong F, et al. Succession and Fermentation Products of Grass Carp (*Ctenopharyngodon idellus*) Hindgut Microbiota in Response to an Extreme Dietary Shift. *Frontiers in Microbiology*. 2017;8:1585. doi:[10.3389/fmicb.2017.01585](https://doi.org/10.3389/fmicb.2017.01585)
47. Hu Y, Liang Y, Han L, et al. Phytogetic diet enhances the growth and disease resistance of the black-spotted frog, *Pelophylax nigromaculatus*, by stabilizing intestinal homeostasis and boosting innate immunity. *Fish & Shellfish Immunology*. 2025;165:110553. doi:[10.1016/j.fsi.2025.110553](https://doi.org/10.1016/j.fsi.2025.110553)
48. Shin JH, Tillotson G, MacKenzie TN, Warren CA, Wexler HM, Goldstein EJC. Bacteroides and related species: The keystone taxa of the human gut microbiota. *Anaerobe*. 2024;85:102819. doi:[10.1016/j.anaerobe.2024.102819](https://doi.org/10.1016/j.anaerobe.2024.102819)
49. Liu S, Li F, Cai Y, et al. Bacteroidaceae, Bacteroides, and Veillonella: emerging protectors against Graves' disease. *Frontiers in Cellular and Infection Microbiology*. 2024;14:1288222. doi:[10.3389/fcimb.2024.1288222](https://doi.org/10.3389/fcimb.2024.1288222)
50. Deng L, Wang S. Colonization resistance: the role of gut microbiota in preventing *Salmonella* invasion and infection. *Gut Microbes*. 2024;16:2424914. doi:[10.1080/19490976.2024.2424914](https://doi.org/10.1080/19490976.2024.2424914)
51. Fenneman AC, Rampanelli E, Yin YS, et al. Gut microbiota and metabolites in the pathogenesis of endocrine disease. *Biochemical Society Transactions*. 2020;48:915-931. doi:[10.1042/BST20190686](https://doi.org/10.1042/BST20190686)
52. Shin N, Whon TW, Bae J. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*. 2015;33:496-503. doi:[10.1016/j.tibtech.2015.06.011](https://doi.org/10.1016/j.tibtech.2015.06.011)
53. Wang S, Liu S, Wang C, et al. Dietary Antimicrobial Peptides Improve Intestinal Function, Microbial Composition and Oxidative Stress Induced by *Aeromonas hydrophila* in Pengze Crucian Carp (*Carassius auratus* var. *Pengze*). *Antioxidants*. 2022;11:1756. doi:[10.3390/antiox11091756](https://doi.org/10.3390/antiox11091756)