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# Lvvibriocin-GK effectively reduced skin ulcer syndrome of *Apostichopus japonicus* by eliminating surface bacteria, modulating gut microbiota, and enhancing host immune responses

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ABSTRACT

Skin ulceration syndrome (SUS) is a major threat to the aquaculture of *Apostichopus japonicus*, particularly in southern China, where it has shown high mortality rates and infectious potential. Traditional antibiotic treatments often lead to challenges such as antibiotic resistance. Antimicrobial peptides (AMPs), which are vital elements of innate immunity, represent a promising alternative for treating SUS. In the study, a novel AMP named Lvvibriocin-GK identified in *Litopenaeus vannamei* was found to have a strong antibacterial activity against multiple *Vibrio* species that possibly cause SUS. Through constructing a *Vibrio harveyi*-induced SUS model, we evaluated the efficacy of a 7-day Lvvibriocin-GK immersion treatment to SUS. Compared to doxycycline hydrochloride at the same concentration, Lvvibriocin-GK treatments could have ulcer area and numbers reduced, mortality decreased, the DAI index significantly lowered, as well as intestinal inflammatory cell infiltration decreased but no significant effect on body weight. The therapeutic effects of Lvvibriocin-GK were accompanied by significantly enhancing the activities of trypsin, lysozyme, T-NOS, and T-SOD and reducing *Vibrio harveyi* load in tissues. qPCR results indicated that Lvvibriocin-GK upregulated the expression of intestinal barrier proteins *ZO-1* and *Occludin*, and downregulated pro-inflammatory factors such as *IL17*, *p105*, *NLRP3*, *Rel*, and *Stat5*. Furthermore, 16S rRNA sequencing revealed that the beneficial effects of Lvvibriocin-GK might be linked to favorable changes in *A. japonicus* 's gut microbiota, including increased microbial diversity, enhanced abundance of potential probiotics (*Rhodobacteraceae*, *Bacillus*, *Serratia liquefaciens*), and reduced the abundance of opportunistic pathogens (*Acinetobacter* and *Bacteroides vulgatus*). These changes resulted in a more complex microbial network and improved immune-associated functions, particularly through pathways such as NF-κB signaling. Mantel tests indicated stronger correlations between Lvvibriocin-GK-treated gut microbiota and disease phenotypes (gut pathology), enzymatic activities (lipase, lysozyme, T-NOS, T-SOD), intestinal barrier markers (*Occludin*), and immune-related genes (*Stat5*, *Rel*, *FoxP*, *VEGF*). Taken together, this study proposes a novel, environmentally friendly AMP immersion treatment for severe cases of SUS. The therapeutic effects are closely to effectively eliminate pathogens, modulate the gut microbiota and enhance host immunity. A comprehensive evaluation of the efficacy and mechanisms of AMP treatment in *A. japonicus* SUS will contribute to assessing its advantages and potential applications as an antibiotic alternative, promoting *A. japonicus* health and improving aquaculture practices.

## 1. Introduction

*Apostichopus japonicus* is highly valued in Asia, particularly in China, for its rich nutritional and medicinal benefits, making it an important marine economic species [1]. In recent years, China's *A. japonicus* production has steadily increased, reaching a record high of 292,508 tons in

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2023 [2]. Fujian Province has emerged as an important region for *A. japonicus* aquaculture in China in recent years, ranking third in national production. However, high-density and intensive farming practices pose significant challenges, particularly the spread of infectious diseases, resulting in substantial economic losses in Fujian Province. Skin Ulceration Syndrome (SUS) is recognized the most prevalent and devastating disease affecting *A. japonicus* farming [3]. Symptoms of SUS in *A. japonicus* include oral swelling, head shaking, decreased adhesion, the appearance of white spots on ulcerated areas, viscera ejection, inability to feed, skin ulceration, and mass mortality [1,4,5]. Studies have revealed that the pathogens causing SUS in China are diverse, among which with bacterial pathogens such as *Vibrio splendidus*, *Vibrio alginolyticus*, *Vibrio. harveyi*, *Shewanella smarisflavi*, and *Pseudoalteromonas tetraodonis* are predominant [3,6–8]. Currently, antibiotics are the primary means of controlling SUS. However, their excessive use exacerbates resistance, undermines sustainable disease management, and threatens both environmental and human health [9,10]. In 2024, the outbreak of SUS in Fujian's *A. japonicus* farms led to rapid weight loss, low survival rates, and significant financial losses, with large amounts of *V. harveyi* and *Vibrio fortis* isolated from infected individuals. To ensure the Fujian's *A. japonicus* industry, it is imperative to explore new strategies that effectively prevent and treat SUS caused by bacterial infections, reducing environmental pollution from antibiotic use.

Antimicrobial peptides (AMPs) play a vital role in the innate immune system, demonstrating a wide range of antibacterial and antifungal properties, including significant effects against antibiotic-resistant strains. Additionally, AMPs can enhance host immunity and promote growth, making them a potential alternative to traditional antibiotics [11]. Numerous studies have confirmed the potential of AMPs in the treatment of human skin diseases, such as Pseudin-2, Scyreptin$_{1\text{-}30}$ and Bolespleenin$_{334\text{-}347}$ [12–14]. However, in aquaculture, AMPs are primarily used as feed additives, where they significantly regulate fish gut microbiota, enhance host immunity, improve disease resistance, and promote growth. For instance, bovine lactoferrin (Lf), cecropin AD, and Tilapia hepcidin 2-3 have been shown to modulate gut microbiota, increase enzyme activity, and improve immune parameters, thereby enhancing fish survival rates when challenged with *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Vibrio alginolyticus* [15–17]. AMP Scy-hepc developed in our lab has significantly enhanced both the growth and disease resistance of *Larimichthys crocea* and *Epinephelus akaara* [18,19]. Moreover, the integration of AMP cathelicidin and cecropin into the genome of channel catfish has been shown to enhance survival rates following infection with *Edwardsiella ictaluri* and *Flavobacterium covae* [20]. However, to our knowledge, the majority of studies have concentrated on utilizing AMPs as feed additives in aquaculture, with limited studies exploring their potential as immersion-based therapeutics for treating SUS. Given that *A. japonicus* suffering from SUS viscera ejection and are unable to feed, administering medications through feed additives becomes challenging. Therefore, inspired by the therapeutic use of AMPs in human skin diseases and the antibiotic treatment of SUS in *A. japonicus*, exploring AMP-based immersion therapy for SUS is highly significant.

To assess the feasibility of AMP-based immersion therapy in aquaculture, this study builds on our laboratory's previously established transcriptome databases of marine animals after bacterial infection. From these data, several novel functional peptides were screened and identified. Further validation through antimicrobial activity assays led to identify a new functional peptide with broad-spectrum antimicrobial activity, particularly showing strong inhibitory effects against various *Vibrio* species in seawater. This peptide, named Lvvibriocin-GK, was derived from the transcriptome database of *Litopenaeus vannamei*. We further developed a *V. harveyi*-induced SUS model in *A. japonicus*, assessing the potential of Lvvibriocin-GK in treating SUS through changes in disease phenotypes, immune indicators, and gut microbiota composition. To our knowledge, most existing probiotic therapies in *A. japonicus* aquaculture are primarily preventive, functioning mainly by modulating the gut microbiota and enhancing host immune responses [1]. And there are currently few therapeutic agents specifically designed for *A. japonicus* severely infected with SUS [1,9]. Moreover, the overuse of antibiotics therapies usually leads to increased bacterial resistance. In this study, we developed an immersion-based treatment strategy using the AMP Lvvibriocin-GK, which not only expands the repertoire of anti-*Vibrio* peptides, but also provides a comprehensive evaluation of the therapeutic effects and underlying mechanisms of Lvvibriocin-GK in treating SUS. This study would provide an ideal candidate substituting for antibiotics for the treatment of severe SUS as immersion-based therapeutics.

## 2. Materials and methods

### 2.1. Experimental strains

The bacterial strains utilized in this study were obtained from the China General Microbiological Culture Collection Center (CGMCC), comprising *Staphylococcus aureus* (CGMCC no. 1.2465), *Staphylococcus epidermidis* (CGMCC no. 1.426), *Vibrio fluvialis* (CGMCC no. 1.1609), *Vibrio harveyi* (CGMCC no. 1.1593), *Vibrio alginolyticus* (CGMCC no. 1.1833), *Pseudomonas fluorescens* (CGMCC no. 1.3202), *Acinetobacter baumannii* (CGMCC no. 1.6769), *Pseudomonas aeruginosa* (CGMCC no. 1.2421), *Salmonella enteritis* (CGMCC no. 1.1859), and *Escherichia coli* (CGMCC no. 1.2389). Additionally, *Photobacterium damsela* was supplied by the Fisheries Research Institute of Fujian, *Streptococcus agalactiae*, *Lactococcus garvieae*, *Vibrio parahaemolyticus*, *Pseudomonas putidae*, and *Aeromonas schubertii* were supplied by the Guangdong HAID Research Institute. *Vibrio* strains were cultivated at 28 °C on marine broth 2216 agar (BD DIFCO, USA), while other bacterial species were cultivated at 37 °C in nutrient broth (OXBID, UK).

### 2.2. Screening, protein sequence analysis and peptides synthesis of Lvvibriocin-GK

Based on the transcriptome database of *L. vannamei* and utilizing machine learning methods established in our laboratory, ten potential AMPs were screened for their antibacterial activity and efficacy in seawater. Among them, the peptide Lvvibriocin-GK exhibited significant anti-*Vibrio* activity. The physicochemical properties of the protein were predicted with ProtParam and HeliQuest. The chemical structure of the peptide was constructed using ChemDraw and its three-dimensional conformation was predicted with AlphaFold and visualized using PyMOL [12]. The peptide was chemically synthesized via solid-phase synthesis by GenScript (Nanjing, China), achieving a purity of 95.56 %, as assessed by reverse-phase high-performance liquid chromatography (RP-HPLC). The molecular weight was determined using electrospray ionization mass spectrometry (ESI-MS), yielding a value of 2.043 kDa.

### 2.3. Antimicrobial activity assay

The antimicrobial activity was evaluated employing microbroth dilution technique, as outlined in previous studies [21]. Bacteria in the log phase of growth were diluted to a concentration of $1 \times 10^{\wedge}6$ CFU/mL in appropriate growth media. In sterile 96-well polystyrene plates, the assay solution was prepared by combining 50 μL of diluted Lvvibriocin-GK with 50 μL of bacterial suspension. Different concentrations of Lvvibriocin-GK were prepared in Milli-Q water, subsequently added to bacterial suspensions in 96-well polystyrene plates, with final peptide concentrations ranging from 1.5 to 96 μM. The control group included bacterial suspensions treated solely with Milli-Q water. The plates were incubated at the optimal growth temperature for each bacterial strain for a duration of 24 h. The minimum inhibitory concentration (MIC), was calculated as the lowest peptide concentration yielding no detectable growth. Each experiment was independently

repeated three times with three parallel replicates per group. The antimicrobial effectiveness of Lvvibriocin-GK against *V. harveyi* in seawater was evaluated with a dynamic shaking method [22]. A single colony of *V. harveyi* was picked and propagated in 2216E liquid medium until the logarithmic growth phase. The bacterial cells were collected, rinsed three times with seawater, subsequently adjusted to a concentration of 1 × 10^3 CFU/mL. Lvvibriocin-GK was introduced into the prepared bacterial suspension at final concentrations of 25 μg/mL, 50 μg/mL, and 100 μg/mL, in sequence. The control group consisted of bacterial suspensions without the addition of Lvvibriocin-GK. All samples were incubated in a shaking incubator at 28 °C and 100 rpm, with plate counts conducted at 24-h intervals to determine the bacteriostatic rate.

### 2.4. Electron microscope ultramicroscopy assay

Scanning electron microscopy was employed to examine the morphological alterations of bacterial cells induced by Lvvibriocin-GK, as reported in prior studies [19]. Bacterial cells during the logarithmic growth phase were harvested and resuspended in NaPB buffer to achieve an approximate concentration of 10^7 CFU/mL. To assess the effect of Lvvibriocin-GK, a final concentration of 3 μM Lvvibriocin-GK was added to the bacterial suspension, which was subsequently incubated at 37 °C for 30 min. Following incubation, bacterial cells were collected by centrifugation (5000×*g*, 5 min), then resuspended in 2.5 % (v/v) glutaraldehyde (Sigma, Germany) for fixation. The suspension was incubated at 4 °C overnight. After fixation, the cells were rinsed with NaPB buffer for three times, subsequently 10 μL of NaPB was incorporated to achieve a concentrated suspension. The suspension was gently applied to poly-L-lysine-coated glass slides, which had been pre-cut, and incubated on ice for 30 min. Excess liquid was absorbed with filter paper, and the samples were subsequently dehydrated through a graded ethanol series. Following dehydration, the samples were critical point dried using an EM CPD300 (Leica, Germany). Finally, the samples were coated with a thin metal layer via an ion sputter coater (JFC-1600, Jeol, Germany) and examined using a scanning electron microscope (SEM, Zeiss SUPRA 55, Germany).

### 2.5. Measurement of peptide thermal stability

Peptide stability was evaluated using a well-established protocol outlined in previous literature [12]. Briefly, *V. harveyi* cell suspensions in the logarithmic growth phase were collected and adjusted to a concentration of 1 × 10^6 CFU/mL. The peptide Lvvibriocin-GK with a final concentration of 3 μM was then subjected to heat treatment in a thermostatically controlled water bath at 100 °C for 10, 20, and 30 min. Following cooling, treated peptides were combined alongside the diluted bacterial suspension in the wells of a microtiter plate and incubated at 37 °C. Absorbance readings at 600 nm were recorded at designated time points with enzymatic microplate reader (Infinite F200 PRO, Tecan, Switzerland) to monitor bacterial growth. Each condition was assessed in triplicate across three independent experiments.

### 2.6. Cytotoxicity assays

Cytotoxicity was assessed following a previously established protocol [13], targeting zebrafish embryonic (ZF4) and human embryonic kidney (HEK 293T) cell lines. ZF4 cells were provided by China Zebrafish Resource Center, while HEK 293T cells were provided by Stem Cell Bank, Chinese Academy of Sciences. Briefly, ZF4 cells were maintained in DMEM/Nutrient Mixture F-12 (Hyclone, No. SH30023.01) containing 10 % FBS and antibiotics (10 U/mL penicillin and 10 μg/mL streptomycin) at 28 °C under the same $CO_2$ conditions. HEK 293T cells were maintained in the Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum (FBS; Gibco, Australia) at 37 °C in a humidified atmosphere with 5 % $CO_2$. Both cell lines were plated at a starting density of 1 × 10^5 cells/mL in 96-well flat-bottomed plates (Thermo Fisher, USA) and incubated overnight under their respective culture conditions. The following day, the culture mediums were substituted with fresh medium supplemented with Lvvibriocin-GK at final concentrations of 0, 6, 12, 24, 48, and 96 μM. After 24 h of culture, cell viability was assessed via CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, USA). Absorbance was measured at 492 nm with a microplate reader (Infinite F200 PRO, TECAN, Switzerland). Each experiment was performed in triplicate.

### 2.7. Preparation of V. harveyi suspension

Given the isolation of a large amount of *V. harveyi* from skin ulceration syndrome (SUS)-affected *A. japonicus* in the Xiapu aquaculture region of Fujian in 2024, this study used the *V. harveyi* strain (purchased from CGMCC, no. 1.1593) for challenge experiments to induce SUS. *V. harveyi* was grown in 2216E marine broth at 28 °C for 12 h, then collected by centrifugation at 4000×*g* and 4 °C for 10 min, rinsed with saline solution, and subsequently resuspended in saline. The bacterial solution was standardized to 1 × 10^8 CFU/mL, and served as the inoculum for infecting the *A. japonicus* in the challenge experiments.

### 2.8. A. japonicus collection and culture

*A. japonicus* with an average weight of 49.42 ± 6.2 g were sourced from Xindonghai Aquaculture Company in Xiapu County, Ningde, Fujian Province, China. After collection, a total of 60 individuals were acclimated in fully aerated seawater tanks (40 × 40 × 100 L) without feeding, with daily water changes. After a one-week acclimation period, the infection challenge experiments were conducted with healthy *A. japonicus*. The water source is drawn from the natural seawater area in Xiapu County and the temperature was kept within the range of 13.2–14.1 °C, salinity between 29.7 and 30.2 ‰, dissolved oxygen levels from 10.5 to 11.5 mg/L, pH maintained at 7.5–7.8. All the aquaculture experiments were finished in Marine Fisheries Development Center of Xiapu.

### 2.9. Experimental design and sample collection

After acclimation, all the *A. japonicus* were transferred to aquaculture tanks containing a 1 × 10^8 CFU/mL suspension of *V. harveyi*, with continuous aeration. To accelerate the infection process, a sterile scalpel was used to create a shallow 1 × 1 $mm^2$ superficial incision on the surface of each *A. japonicus*, facilitating bacterial entry and speeding up infection. After 24 h of *V. harveyi* infection, the condition of all *A. japonicus* was assessed. A total of 45 *A. japonicus* were then sampled randomly and assigned into three groups, including AMP Lvvibriocin-GK group, doxycycline hydrochloride group and control group, with 15 individuals in each group. Each group was further subdivided into three replicates, with 5 individuals per replicate. Both AMP Lvvibriocin-GK and doxycycline hydrochloride (Weifang Zhonghe Animal Pharmaceutical Co., Ltd.) were reconstituted in seawater to a concentration of 25 μg/mL. *A. japonicus* in the treatment group were removed daily for a 20-min immersion in the AMP solution and then returned to fresh, fully aerated seawater. The control group was immersed in fresh seawater following the same procedure. The treatment lasted for 7 days, with water changes twice daily. After treatment, disease status and body weight were assessed. *A. japonicus* were rinsed with sterile seawater, dissected using sterile tools, and samples of the hind gut and body wall were collected. Additionally, 50 μL of coelomic fluid was extracted from each sea cucumber, pooled, and combined with an equivalent volume of anticoagulant.

### 2.10. Bacterial load assay

Bacterial load determination was conducted following previously described methods [23]. After 24 h of *V. harveyi* infection, the

respiratory tree and body wall tissues of *A. japonicus* from both the 7-day AMP treatment group, 7-day doxycycline hydrochloride treatment group and the control group were collected under sterile conditions (n = 6). Each tissue sample was processed by homogenization in 1 mL of sterile PBS. The homogenate was subsequently serially diluted in sterile PBS and transferred onto individual 2216E Agar plates. Following a 24-h incubation at 28 °C, colony enumeration was carried out for each sample to determine the bacterial load.

### 2.11. Disease activity index assay

Based on vertebrate enteritis models, such as mice and fish, and considering the infection characteristics of *A. japonicus* SUS, a disease activity index (DAI) evaluation system was established [24,25]. This system includes indicators such as viscera ejection, oral swelling, head shaking, ulceration, attachment capability, contraction, and autolysis. Detailed evaluation criteria and the DAI scores of 15 individuals in each group are provided in Table S1.

### 2.12. Enzyme activity index assay

Weigh the intestinal tissues accurately and prepare intestinal homogenates. For measuring trypsin activity, use a sample-to-volume ratio of 1:9 (weight in grams to volume in milliliters), adding the sample to nine times its volume of homogenization medium (0.9 % saline). For measuring lipase activity, prepare a 20 % homogenate with a 1:4 sample-to-medium ratio. Homogenize the samples mechanically in an ice-cold water bath, centrifuge at 2500 rpm for 10 min, and then carefully collect the resulting supernatant (10 % or 20 % homogenate supernatant) for further analysis. Centrifuge the body cavity fluid at 10,000 rpm for 10 min, resuspend the pellet in isotonic body cavity cell solution, and sonicate on ice for 5 min (Body cavity cell isotonic solution: 0.001 M EGTA, 0.34 M NaCl, 0.01 M Tris-HCl, pH 8.0). Obtain the final body cavity cell lysate supernatant for analysis. Use the lipase assay kit (No. A054-1-1), trypsin assay kit (No. A080-2), and lysozyme assay kit (No. A050-1-1) from Nanjing Jiancheng Bioengineering Institute to measure the lipase, trypsin, and lysozyme activities in *A. japonicus* intestinal homogenates following manufacturer's instructions. For total nitric oxide synthase (T-NOS) activity, use the assay kit (No. A014-2), and for superoxide dismutase (T-SOD) activity, use the assay kit (Catalog No. A001-1) from Nanjing Jiancheng Bioengineering Institute, according to the manufacturer's instructions to measure total NOS activity in *A. japonicus* body cavity cell lysate. Measure total SOD activity in *A. japonicus* body cavity cell lysate using the hydroxylamine method. 5 individuals were sampled randomly from each group.

### 2.13. Quantitative real-time PCR analysis

The expression of immune-related genes was measured in the posterior gut, respiratory trees, and longitudinal muscles of *A. japonicus* using qRT-PCR. RNA samples for qRT-PCR were prepared from three biological replicates. Gene expression levels were quantified via the comparative CT method (2ˆ-ΔΔCT) with specific primers from published researches (Table S2) [26]. qRT-PCR was performed with an Applied Biosystems 7500 instrument (Life Technologies, USA), while the resulting data were processed using 7500 System SDS software. The experiments were repeated a minimum of twice. Data are presented as mean ± standard deviation (n = 5). Statistical significance between groups was assessed via a two-tailed Student's t-test, while $P$ values < 0.05 regarded as significant.

### 2.14. Histopathological analysis

Hematoxylin and eosin (H & E) staining was used to examine the intestinal and body wall tissues of *A. japonicus* infected with SUS following treatment. The tissue samples were initially immersed in 4 % paraformaldehyde and subsequently sectioned. The sections from both the intestine and body wall tissues were stained with H&E according to previously established methods [27]. The stained sections were analyzed using light microscopy (Eclipse 50i; Nikon, Tokyo, Japan).

### 2.15. DNA extraction

Genomic DNA was extracted from each sample using the method described in our previous study [28], with the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), ensuring compatibility with the subsequent sequencing protocols.

### 2.16. S rRNA sequencing of the gut microbiota

The V3-4 region, amplified by PCR, was chosen for sequencing using the Illumina PE250 platform. Sequencing was carried out at Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China). The primers, experimental conditions, protocols, and kits used in this study were in accordance with those outlined in the previous study [29], resulting in the construction of the final amplicon library.

### 2.17. Sequence data processing

The microbial bioinformatics analysis was performed following the methodology outlined in a prior investigation [29]. In brief, paired-end raw sequence reads were analyzed via DADA2 package (version 1.14) [30], which involved merging, filtering, denoising, chimera removal, and clustering into amplicon sequence variants (ASVs). The classified ASVs were analyzed using a naive Bayesian model and the RDP classifier (version 2.2), with taxonomy assignments derived from SILVA database (version 138.1) and the confidence threshold of 0.8.

### 2.18. Bioinformatics analysis

Processed ASV datas were used to calculate α-diversity metrics, including Chao index, with QIIME software package [31]. Bray–Curtis dissimilarities were applied to assess β-diversity, followed by principal coordinate analysis (PCoA) using vegan package in R [31]. Indicator species analysis was conducted via the labdsv and indicspecies packages within R [32]. Key microbial taxa were identified using the LEfSe software (version 1.0) [33], with a linear discriminant analysis (LDA) score threshold of 3.5. Co-occurrence networks were constructed to investigate the relationships among microbial communities in *A. japonicus*. Network analysis was carried out via the Hmisc, vegan, and igraph packages in R [34], and the correlation networks were depicted with Gephi software [35]. Network robustness was determined and assessed with WGCNA package in R. Effect sizes for continuous variables were calculated using Cohen's d, implemented via the effsize package in R. All reported effect sizes showed absolute values.

All statistical analyses were performed in R (version 3.5.1, R Development Core Team) unless otherwise specified.

## 3. Results

### 3.1. Lvvibriocin-GK information and prediction

Here, a potential antimicrobial peptide was discovered from *L. vannamei*. Which consists of 17 amino acids with a molecular weight of approximately 2.043 kDa, including five positively charged residues. According to predictions, this AMP has an isoelectric point of 10.30 and a hydrophobicity of 41 %, classifying it as a amphiphilic cationic antimicrobial multiple peptides, named Lvvibriocin-GK (GKKVWKKYYFVLRASGL). And the predicted tertiary structure of Lvvibriocin-GK adopts a typical α-helix configuration, with its amino acid composition dominated by basic residues. The hydrolyzable groups on the side chains (guanidino and ε-amino groups) contribute to the

cationic nature of the peptide. Lvvibriocin-GK is predicted to have a net charge of +5 and a hydrophobicity of 41 %. Subsequently, an AMP probability analysis was conducted using CAMPR4 server. The random forest, support vector machine, and artificial neural network models yielded probabilities of 1.00,1.00 and 0.99. All three models identified Lvvibriocin-GK as a potential AMP (Fig. 1).

### 3.2. Broad-spectrum antibacterial activity of Lvvibriocin-GK

The antimicrobial assay results demonstrated that Lvvibriocin-GK exhibits broad-spectrum antimicrobial activity (Table 1), particularly showing significant inhibitory effects against multiple *Vibrio* species commonly found in aquatic pathogens, including *V. fluvialis*, *V. parahaemolyticus*, *V. harveyi*, and *V. alginolyticus*, with a MIC range of 1.5 μM–6 μM. Most gram-negative and gram-positive bacteria exhibited susceptibility to this peptide. These findings indicate that Lvvibriocin-GK possesses broad-spectrum antibacterial activity (Table 1). Furthermore, dynamic shaking method analysis evaluated Lvvibriocin-GK's inhibitory effect on *V. species* in seawater, revealing that 25 μg/mL Lvvibriocin-GK was able to continuously kill more than 95 % of *V. harveyi* over a 5-day period (Fig. S1).

We further assessed the impact of Lvvibriocin-GK on the bacterial surface structure of *V. harveyi* using scanning electron microscopy (SEM). These results demonstrated that the membrane surface of the bacteria in the control group remained smooth and undamaged. However, following treatment with Lvvibriocin-GK at the concentration of 1 × MIC, the membrane surface of *V. harveyi* exhibited pronounced wrinkling, with severe cases displaying membrane rupture (Fig. 2a). Further investigations demonstrated that Lvvibriocin-GK maintained good antibacterial activity across various temperatures (Fig. 2b). The cell viability of those exposed to Lvvibriocin-GK at concentrations between 6 and 96 μM showed no significant differences when compared to the untreated controls, indicating that Lvvibriocin-GK does not induce cytotoxicity effects on aquatic animal cells. (Fig. 2c).

### 3.3. Lvvibriocin-GK immersion treatment significantly alleviates symptoms of SUS

Given the isolation of a large quantity of *V. harveyi* from *A. japonicus* farming areas affected by skin ulceration syndrome (SUS) in Fujian, we first established a model of SUS induced by *V. harveyi*. This model successfully produced severe SUS symptoms within 24–48 h (Fig. S2). To ensure both the efficacy and timeliness of treatment, and to align with the characteristics and progression of SUS in real-world aquaculture conditions, we initiated Lvvibriocin-GK immersion treatment 24 h post-infection, at which point distinct SUS symptoms had become apparent (Fig. 3a). The results showed that 24 h after *A. japonicus* was infected with SUS, its body weight significantly decreased due to factors such as

**Table 1**
Antimicrobial activities of Lvvibriocin-GK.

| Strains | CGMCC NO | MIC (μM) |
|---|---|---|
| **Gram-positive bacteria** | | |
| *Streptococcus agalactiae* | – | 6–12 |
| *Lactococcus garvieae* | – | 3–6 |
| *Staphylococcus aureus* | 1.2465 | 6–12 |
| *Staphylococcus epidermidis* | 1.426 | 3–6 |
| **Gram-negative bacteria** | | |
| *Vibrio fluvialis* | 1.1609 | <1.5 |
| *Vibrio harveyi* | 1.1593 | 1.5–3 |
| *Vibrio alginolyticus* | 1.1833 | 3–6 |
| *Vibrio parahemolyticus* | – | 3–6 |
| *Photobacterium damselae* | – | 6–12 |
| *Pseudomonas fluorescens* | 1.3202 | 1.5–3 |
| *Pseudomonas putida* | – | 1.5–3 |
| *Acinetobacter baumannii* | 1.6769 | 1.5–3 |
| *Aeromonas schubertii* | – | 6–12 |
| *Pseudomonas aeruginosa* | 1.2421 | 3–6 |
| *Salmonella enteritis* | 1.1859 | 1.5–3 |
| *Escherichia coli* | 1.2389 | 3–6 |

viscera ejection. After 7 days of Lvvibriocin-GK immersion treatment, the mortality rate was reduced (Fig. S3), and body weight remained stable, whereas the control group showed a significant decline in weight (Fig. 3b). Lvvibriocin-GK immersion treatment significantly lowered the disease activity index (DAI) of the *A. japonicus*, while the DAI of the control group increased (Fig. 3c–Table S1). Lvvibriocin-GK treatment also significantly reduced the number and size of ulcers on the *A. japonicus*'s body surface, particularly in the 0–0.25 $mm^2$ (untreated group: 84.4 ulcers; Lvvibriocin-GK-treated group: 36.6 ulcers) and 2–3 $mm^2$ categories (untreated group: 1.2 ulcers; Lvvibriocin-GK-treated group: 0 ulcers), while the control group exhibited an increase in ulcer size and number, especially in those greater than 20 $mm^2$ (Fig. 3d, $P < 0.05$). Additionally, bacterial load analysis revealed that Lvvibriocin-GK treatment notably lowered the bacterial load in the respiratory trees relative to control group, although no marked variation was observed in the body wall (Fig. 3e). At the same concentration, Lvvibriocin-GK treatment exhibited better therapeutic efficacy against the SUS phenotype in *A. japonicus* compared to doxycycline hydrochloride treatment (Fig. S4). These findings indicate that Lvvibriocin-GK has a notable therapeutic effect on SUS, promoting ulcer healing, as evidenced by phenotypic improvements.

### 3.4. Beneficial effects of Lvvibriocin-GK immersion treatment on immune-related parameters in A. japonicus

We further investigated the intestinal and body wall morphology of *A. japonicus* after 7 days of Lvvibriocin-GK immersion treatment, and performed histological analysis of the intestinal and body wall sections

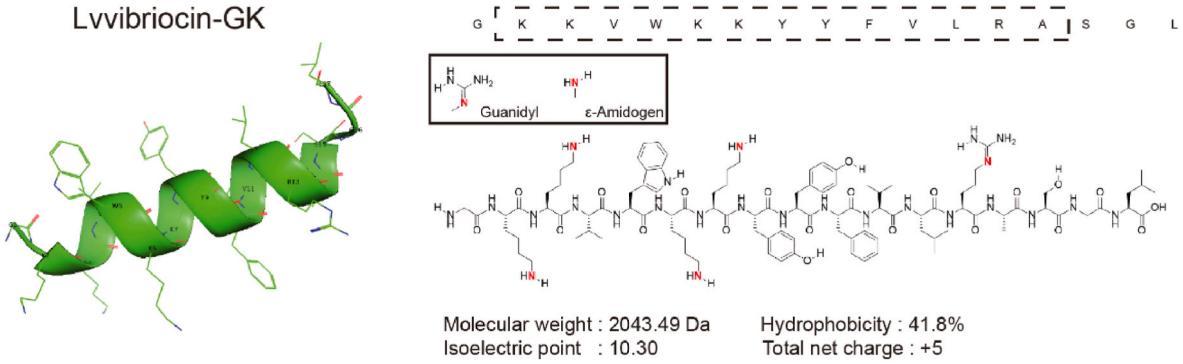


**Fig. 1.** Analysis of the structure and physicochemical properties of Lvvibriocin-GK. The three-dimensional structure of Lvvibriocin-GK was predicted using AlphaFold. The α-helix, which constitutes part of the amino acid sequence, is indicated by a dashed line. The structure also contains basic side chains, with positively charged groups highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

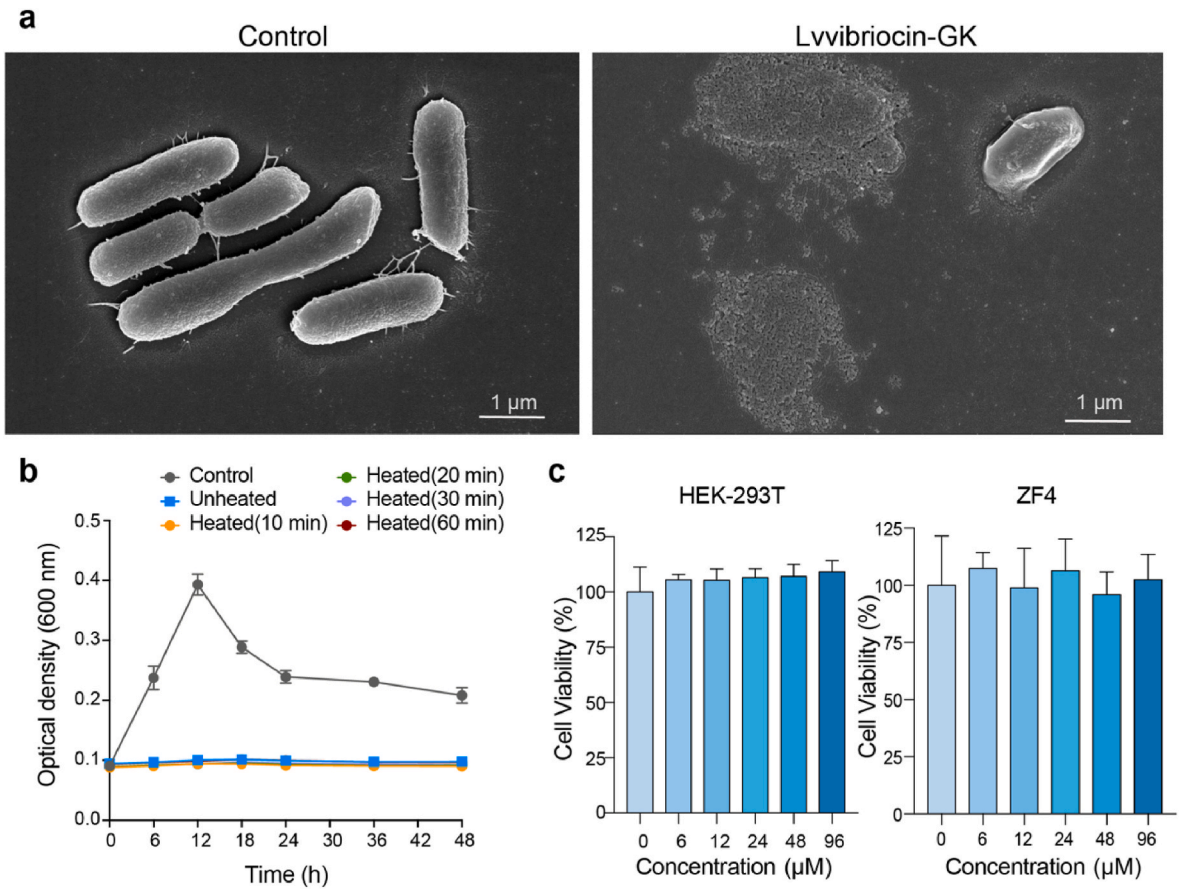


**Fig. 2.** Analysis of the structure effects, stability evaluation and cytotoxicity effects of Lvvibriocin-GK. **a,** structural changes of *V. harveyi* surface in the presence of AMP Lvvibriocin-GK as observed by scanning electron microscopy. **b**, the fluctuation of antimicrobial activity of Lvvibriocin-GK against *V. harveyi* after high temperature treatment. **c,** cytotoxicity of Lvvibriocin-GK on human embryonic kidney cell (HEK-293T) and zebrafish (*Danio rerio*) embryonic cell (ZF4) were assessed using the MTS-PMS assay.

from both the Lvvibriocin-GK-treated and control groups (Fig. 4). These results demonstrated that, in comparison to the control group, the Lvvibriocin-GK-treated group exhibited fewer infiltrating inflammatory cells in the intestines, a reduction in goblet cells, and more intact intestinal morphology, with significantly fewer inflammation-positive areas. Regarding the body wall, no marked variation was observed between Lvvibriocin-GK-treated and control groups. Enzyme activity analysis revealed that Lvvibriocin-GK treatment notably enhanced the activity of trypsin, lysozyme, T-NOS, and T-SOD, while reducing lipase activity (Table 2).

Additionally, relative quantitative analysis showed that after Lvvibriocin-GK immersion treatment, *Zo-1* and *Occludin* were significantly upregulated in the intestine (Fig. 5a), indicating that Lvvibriocin-GK effectively restored the mucosal barrier function. *CAT* expression was significantly downregulated, while *SOD* showed a downward trend but was not statistically significant (Fig. 5b). Other immune-related genes, such as *IL17*, *p105*, *VEGF*, *TGFβ*, *NLRP3*, *Rel*, *FoxP*, and *Socs2*, were also significantly downregulated, while *MMIF* showed no significant difference (Fig. 5c). These results indicate that Lvvibriocin-GK immersion treatment significantly modulate immune-related gene expression in *A. japonicus*, effectively treating SUS.

### 3.5. Beneficial effects of Lvvibriocin-GK immersion treatment on the gut microbiota of A. japonicus

Given that only 40 % of *A. japonicus* in the SUS model did not viscera ejection (7 individuals in Lvvibriocin-GK group and 5 individuals in control group), we randomly chosed 5 individuals from the Lvvibriocin-GK-treated group and all individuals from the control group to assess the impact of Lvvibriocin-GK immersion treatment on gut microbiota (Table S3). Species composition and SIMPER analysis revealed that the shifts in core dominant bacteria between Lvvibriocin-GK-treated and control groups were primarily concentrated in *Proteobacteria*, *Verrucomicrobiota*, *Actinobacteriota*, *Firmicutes* and *Bacteroidota* at the phylum level (contribution rate >1 %, Fig. 6a, Table S4). At the family level, the shifts were mainly seen in *Rubritaleaceae*, *Rhodobacteraceae*, *Comamonadaceae*, *Burkholderiaceae*, *Halomonadaceae*, *Lactobacillaceae*, *Holosporaceae*, *Micrococcaceae*, *Bacteroidaceae*, *Moraxellaceae* and *Nitriliruptoraceae* (contribution rate >1 %, Fig. S5a, Table S4). At the genus level, the primary changes were observed in *Other*, *Rubritalea*, *Pelomonas*, *Ralstonia*, *Halomonas*, *Lactobacillus*, *Nesterenkonia*, *Bacteroides* and *Acinetobacter* (contribution rate >1 %, Fig. S5b, Table S4). Lvvibriocin-GK immersion treatment notably enhanced the α-diversity of the gut microbiota in *A. japonicus* compared to the control group, particularly the Chao index, and the Lvvibriocin-GK group had a greater number of unique ASVs ($P < 0.05$, Fig. 6b–c). We further screened differential ASVs through indval index and random forest analysis, finding that ASV8 (*Rubritalea*), ASV19 (*Rhodobacteraceae*), ASV24 (*Loktanella*), ASV29 (*Bacillus*), and ASV57 (*Serratia liquefaciens*) were significantly enriched in the Lvvibriocin-GK immersion treatment group, most of which are potential probiotics. In contrast, ASV33 (*Acinetobacter*), ASV48 (*Halomonas*), and ASV233 (*Bacteroides vulgatus*) were enriched in the control group, many of which are opportunistic pathogens (Fig. 6d–e). These findings suggest that Lvvibriocin-GK immersion treatment may exert its effects by increasing the abundance of potential probiotics while reducing the abundance of opportunistic pathogens.

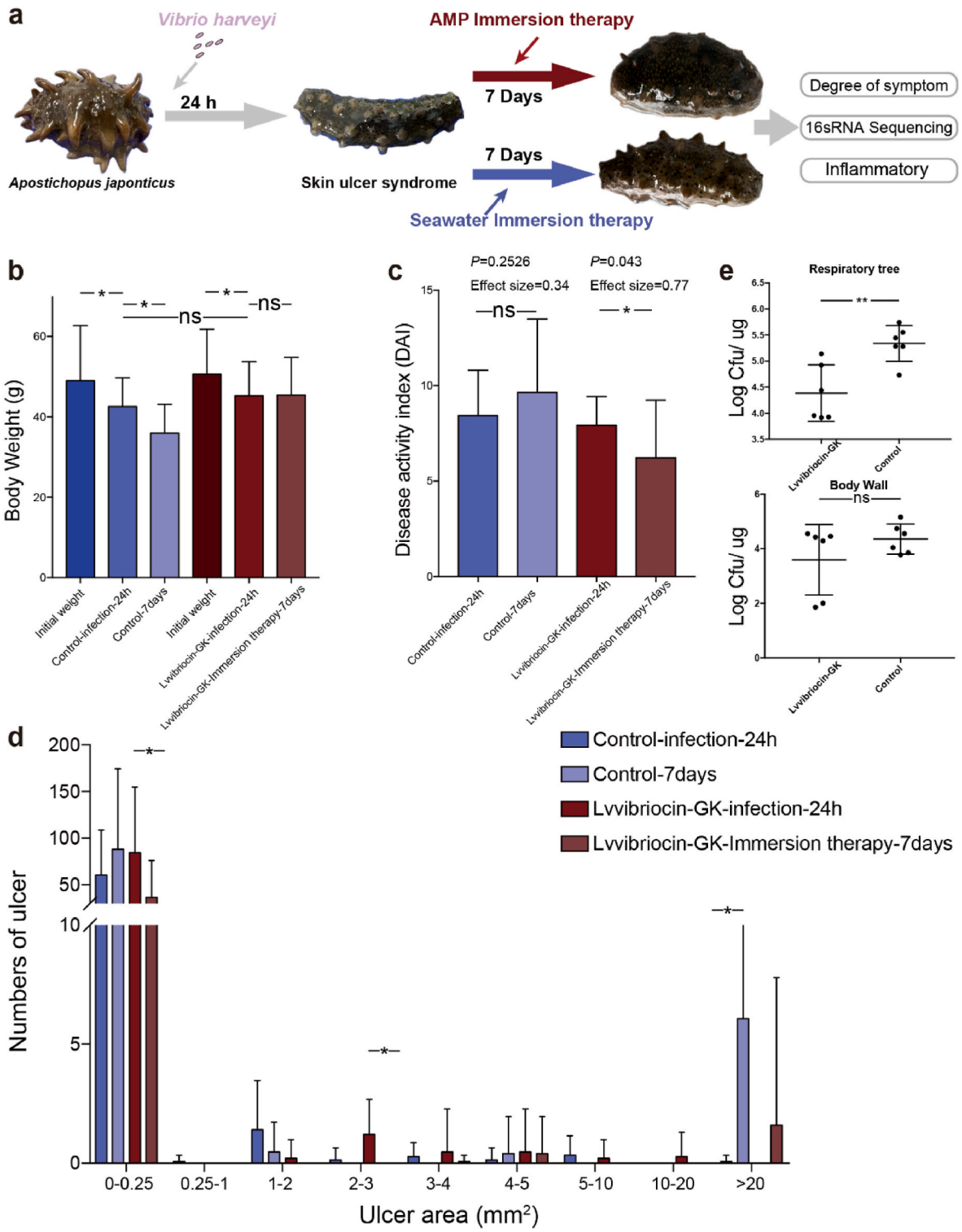


**Fig. 3.** AMP Lvvibriocin-GK immersion treatment significantly alleviates the symptomatic phenotypes of SUS. **a**, the experiment design flow chart of AMP Lvvibriocin-GK immersion treatment in *A. japonicus*. **b**, **c**, **d**, **e**, the body weight (g), disease activity index, bacterial load, the numbers of ulcer and ulcer area between Lvvibriocin-GK immersion group and control group over 7 days treatment after 24 h *V. harveyi* infection. Differences were assessed by two-tailed Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, denoted no significant difference.

LEfSe analysis further confirmed this phenomenon, identifying 54 key species significantly enriched in the Lvvibriocin-GK-treated group, including various potential probiotics like *Bacillus*, *S. liquefaciens*, and *Cellvibrio* (Fig. 6f). Meanwhile, the control group was enriched with 11 key species, the majority of which were opportunistic pathogens. Principal coordinates analysis (PCoA) further evaluated differences in taxonomic composition between the treatment and control groups, revealing significant distinctions between them (Fig. 7a).

We further constructed molecular ecological networks (MENs) to study the interactions among gut microbial species following a 7-day Lvvibriocin-GK immersion treatment, in comparison to the control group (Fig. 7b). In general, the ecological networks between the

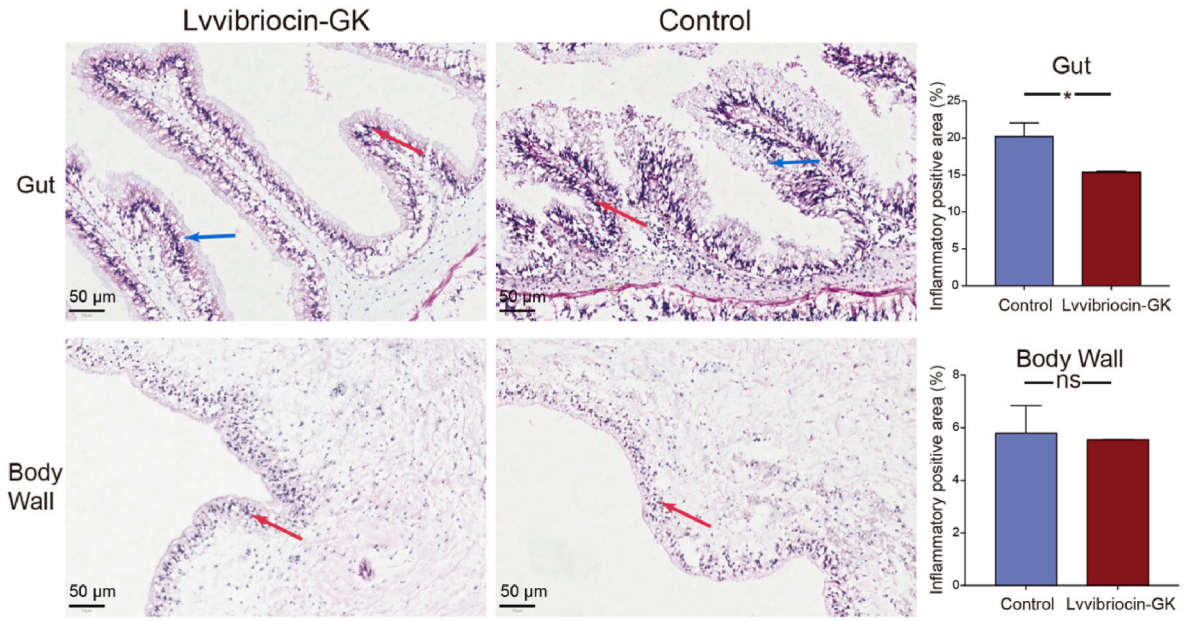


**Fig. 4.** Histological pathology of hind gut and body wall tissues in Lvvibriocin-GK immersion treatment and control group. The blue arrowpoint towards the goblet cells; the red arrowpoint towards the infiltrating inflammatory cells. *$P < 0.05$; **$P < 0.01$; ns, denoted no significant difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**
Enzyme activity of A. japonicus after challenge with V. harveyi after Lvvibriocin-GK immersion treatment.

| Enzyme activity | Lvvibriocin-GK | Control | P value | Effect sizes |
|---|---|---|---|---|
| Lipase (U/g prot) | 117.78 ± 34.8 | 261.97 ± 35.64 | $P$ = 0.0149 | 3.73 |
| Trypsin (U/g prot) | 6994.31 ± 705.57 | 4542.91 ± 352.46 | $P$ = 0.0117 | 3.58 |
| Lysozyme (U/Ml) | 346.86 ± 16.39 | 140.06 ± 55.14 | $P$ = 0.0071 | 4.15 |
| T-NOS (U/mL) | 3.56 ± 0.031 | 2.45 ± 0.15 | $P$ = 0.0005 | 8.13 |
| T-SOD(U/mL) | 0.38 ± 0.065 | 0.12 ± 0.05 | $P$ = 0.0115 | 3.62 |

Lvvibriocin-GK and control group differed significantly. The Lvvibriocin-GK-treated group displayed significantly higher network complexity than control group, as evidenced by the considerable abundance of ASVs. Random removal robustness analysis further confirmed the increased complexity and stability of the network in Lvvibriocin-GK treatment group ($P < 0.05$, Fig. 7c). In both groups, the proportion of positive correlations was markedly higher than that of negative correlations, with more than 73 % of the associations showing positive correlations (Table S5). The bacterial community in the Lvvibriocin-GK immersion treatment group exhibited stronger phylogenetic relationships compared to the control group, with robust interactions between key species, including *Proteobacteria*. These strong interactions may play a role in supporting host health.

Additionally, Picrust functional prediction analysis revealed that Lvvibriocin-GK immersion treatment significantly enhanced immune-related functions (Fig. S6). Specifically, Lvvibriocin-GK immersion treatment resulted in a significant rise in the abundance of pathways such as complement and coagulation cascades, clavulanic acid biosynthesis, VEGF signaling pathway, NF-kappa B signaling pathway, leishmaniasis, transcriptional misregulation in cancer (Fig. S6).

### *3.6. The modulation of gut microbiota and the enhancement of host immunity may be effective mechanisms of Lvvibriocin-GK immersion therapy*

The interplay between gut microbiota and host immunity plays a key role in determining treatment outcomes. Therefore, we further investigated whether factors influencing and characterizing host immunity affect gut microbiota communities, aiming to reveal the interactions between microbial communities and the host immunity. Mantel test results indicated a notable correlation between host immunity and the microbial communities in both the Lvvibriocin-GK immersion treatment group and the control group, with some similarities observed. Specifically, the microbial communities in the Lvvibriocin-GK treatment group were significantly correlated with disease phenotype (gut pathology), intestinal barrier indicators (*Occludin*), enzyme activity markers (lipase, lysozyme, T-NOS, T-SOD), peroxidase-related genes (*CAT*), and immune-related genes (*Stat5*, *Rel*, *FoxP*, *VEGF*), showing a stronger correlation than the control group (Fig. 8). On the other hand, the microbial communities in the control group were notably correlated with disease phenotype (DAI index), intestinal barrier indicators (*Zo-1*), enzyme activity markers (trypsin), and immune-related genes (*NLRP3*), with a stronger correlation observed. These findings suggest that Lvvibriocin-GK immersion therapy may be an effective approach for treating SUS by modulating gut microbiota and enhancing host immunity.

## 4. Discussion

In China, skin ulceration syndrome (SUS) in *A. japonicus* is challenging due to the regional and diverse nature of its pathogens, and effective preventive and therapeutic measures are still lacking [3,8]. In *A. japonicus* aquaculture, broad-spectrum antibiotics are primarily relied upon for prevention and treatment for SUS. Common practices include using antibiotics such as florfenicol, doxycycline hydrochloride for immersion treatment during infections. Additionally, antibiotics are used for immersion before and after transportation to enhance antibacterial capability and improve survival rates. However, long-term overuse of antibiotics can disrupt the ecological balance of the aquaculture environment and alter the gut microbiota of *A. japonicus*, which in turn

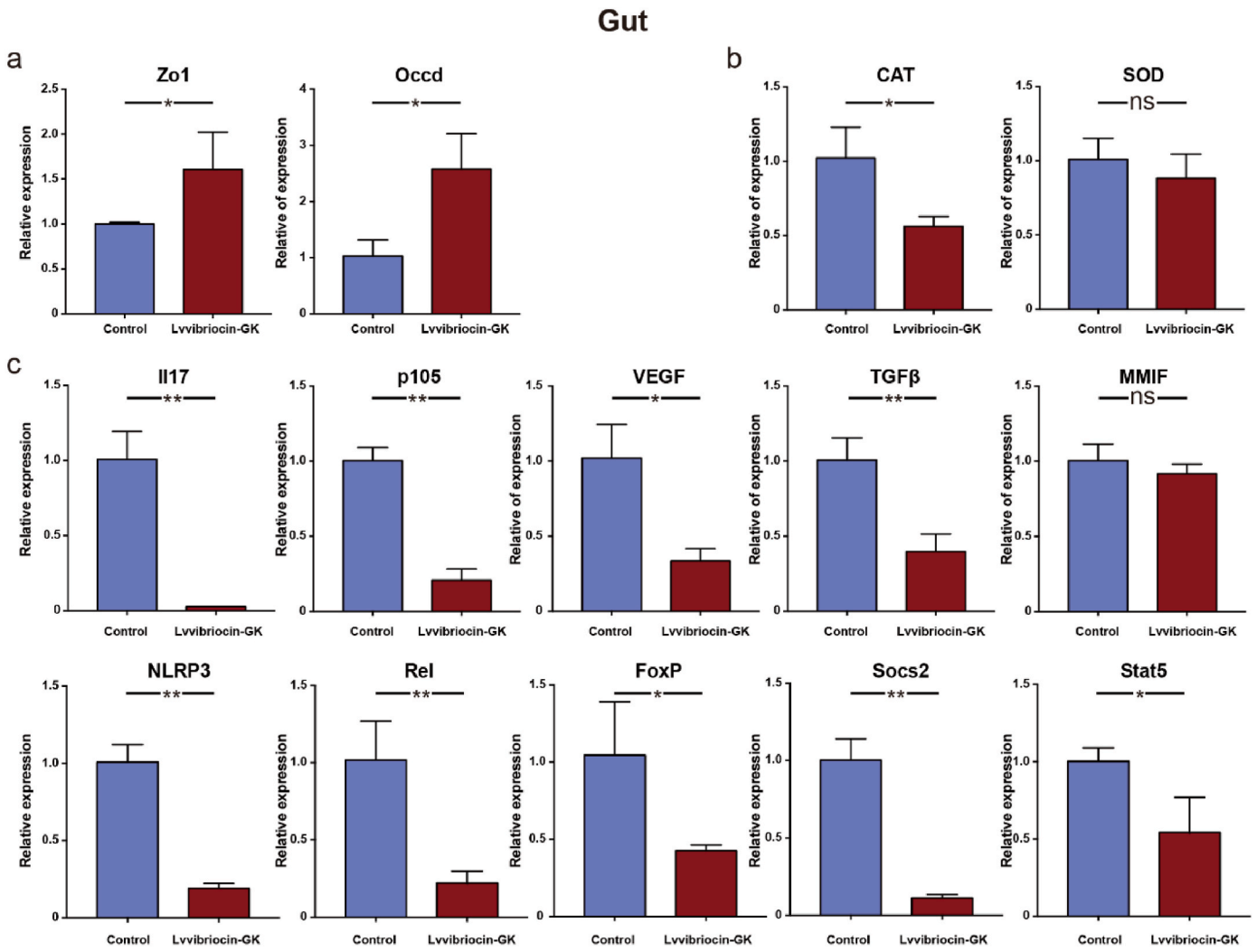


**Fig. 5.** The relative expression of immune-related genes between Lvvibriocin-GK immersion group and control group over 7 days treatment after 24 h *V. harveyi* infection in hind gut of *A. japonicus*. The relative expression of **a**, tight junction protein-related genes, **b**, peroxidase-related genes, **c**, other immune-related genes. Differences were assessed by two-tailed Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, denoted no significant difference.

weakens immunity [36]. This practice also promotes bacterial resistance and leads to antibiotic residues in aquatic products [10]. Given the need to reduce or eliminate antibiotic use, developing effective alternatives has become a prominent research focus both domestically and internationally.

To our knowledge, current research on alternatives to antibiotics for treating SUS primarily focuses on preventive measures through feed or water environment interventions. These approaches include adding chicken egg yolk immunoglobulins (IgY), probiotics, and herbal medicines to feed, and evaluating their efficacy in enhancing *A. japonicus*' resistance to SUS infection [1,9]. Another strategy involves introducing probiotics and other environmental modulators into the water to assess their effectiveness in combating SUS infection at early stages [4]. However, research specifically targeting the development of therapeutic agents for *A. japonicus* already severely infected with SUS is relatively scarce. This study is innovative in its focus on developing a aquaculture medicine specifically for immersion treating SUS in *A. japonicus*, utilizing environmentally friendly alternatives to antibiotics. This represents a challenging strategy.

In 2024, a large-scale outbreak of SUS occurred in *A. japonicus* aquaculture in Fujian Province, with numerous *V. harveyi* isolates identified in the affected *A. japonicus*. Based on this, we established a SUS model induced by *V. harveyi* infection. We then conducted an evaluation criteria by disease activity index (DAI) from enteritis disease models and incorporated specific SUS characteristics, including viscera ejection, oral swelling, head shaking, ulceration, attachment capability, contraction and autolysis [25]. By integrating changes in body weight, bacterial load, ulcer area, immune-related indicators, and gut microbiota, we aimed to better quantify and reflect the progression of SUS. Using these criteria, we further assessed the efficacy of the AMP Lvvibriocin-GK, which targets multiple *Vibrio* species, in treating SUS. The study found that, 24 h after SUS infection, a daily 20-min immersion in Lvvibriocin-GK for 7 days significantly reduced the DAI, stabilized body weight, and notably decreased both the area and number of ulcers on the body surface. These results suggest that AMP Lvvibriocin-GK immersion effectively alleviates the clinical symptoms of SUS. Furthermore, we compared the therapeutic effects of doxycycline hydrochloride at the same concentration. The results indicate that Lvvibriocin-GK exhibits superior efficacy in treating SUS compared to doxycycline hydrochloride, which may be attributed to the lower dosage of doxycycline hydrochloride used.

Previous studies have indicated a link between SUS and damage to the intestinal barrier [37]. Our findings reveal that Lvvibriocin-GK immersion therapy effectively restores the mucosal barrier function of *A. japonicus* by upregulating the expression of *Zo-1* and *Occludin*, while decreasing the infiltration of inflammatory and goblet cells. This effect is

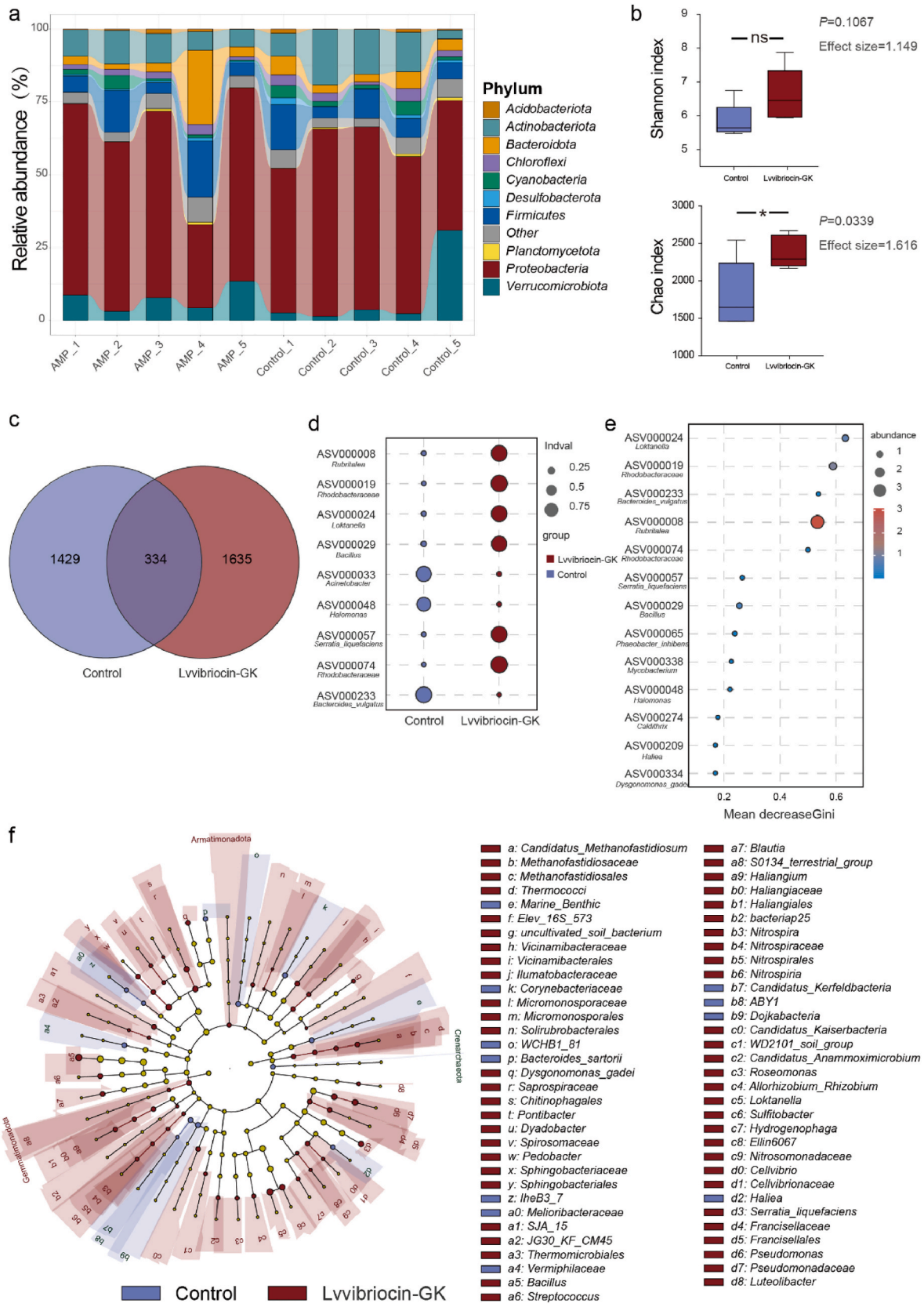


**Fig. 6.** Effects of Lvvibriocin-GK immersion treatment on the gut microbiota of *A. japonicus*. **a**, the divergence of microbial communities between Lvvibriocin-GK immersion treatment and control group, with the relative abundance. Only the dominant microbial phylum with top 10 of each group are plotted. **b**, α-diversity with Shannon and Chao index are plotted. Differences were assessed by two-tailed Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.01$; ns, denoted no significant difference. **c**, venn diagram showing shared or unique ASVs between Lvvibriocin-GK immersion treatment and control group. **d**, indicator species and **e**, random forest analysis between Lvvibriocin-GK immersion treatment and control group. **f**, cladogram generated from linear discriminant analysis (LDA) effect size (LEfSe) showing the relationship between taxon (the levels represent, from the inner to outer rings, phylum, class, order, family, and genus) in four gut parts respectively.

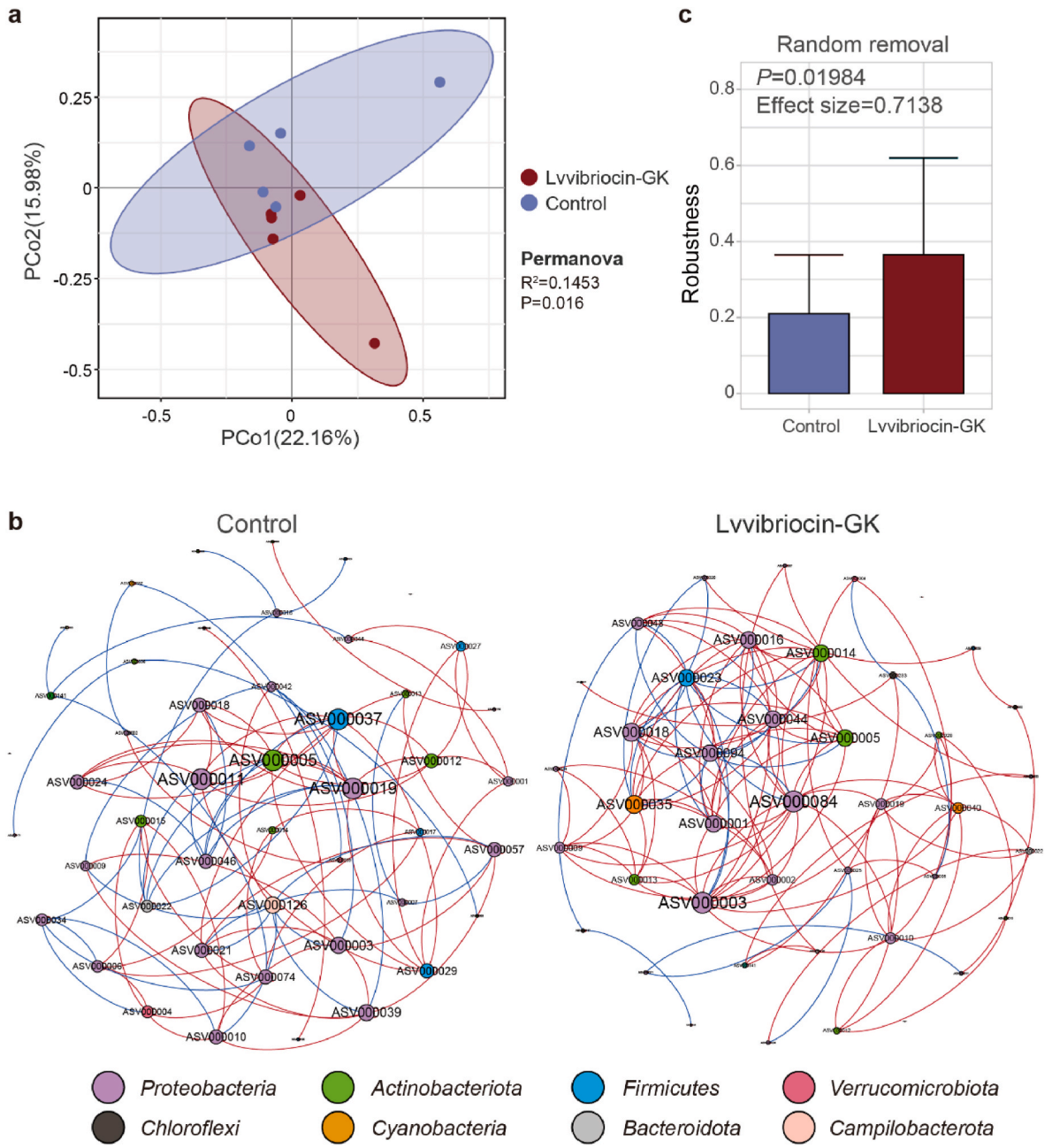


**Fig. 7.** Effects of Lvvibriocin-GK immersion treatment on the gut microbiota of *A. japonicus*. **a**, PCoA plot was constructed using ASV metrics derived from Bray–Curtis dissimilarities. Each point represents a sample. Differences were evaluated using ANOSIM. **b**, co-occurring networks of bacterial ASVs in *A. japonicus* between Lvvibriocin-GK immersion treatment and control group were delineated through correlation analysis. Blue edges signify negative interactions between two nodes, whereas red edges denote positive interactions. **c**, the robustness measured as the proportion of taxa remained with 50 % of the taxa randomly removed from each of the co-occurrence networks. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

comparable to the use of probiotics to improve gut microbiota and restore intestinal epithelial integrity [38]. Additionally, studies have shown that *Vibrio* infection can significantly activate the immune defense responses in marine animals [39]. Our findings reveal that Lvvibriocin-GK immersion therapy significantly enhances enzyme activity and the regulation of immune-related genes, thereby promoting the immune response of *A. japonicus*. Specifically, the activity of trypsin, lysozyme, T-NOS, and T-SOD increased significantly. Among them, lysozyme plays a key role by hydrolyzing bacterial cell wall peptidoglycans and exerting antimicrobial, antiviral, anti-inflammatory, and immune-enhancing effects [40]. Antioxidant enzymes like SOD and CAT help to eliminate the excess of ROS, preventing cellular toxicity [41]. The increase in these enzyme activities significantly enhances the host's ability to resist bacterial infections. Notably, although T-SOD levels were significantly elevated, relative quantification results showed a significant downregulation of peroxidase-related genes (*SOD* and *CAT*). This discrepancy may be attributed to the complex immune regulatory processes within the host and differences in tissue-specific responses to *SOD* and *CAT*. Overall, Lvvibriocin-GK immersion therapy tends to downregulate pro-inflammatory immune factors. For instance, in the intestine, Lvvibriocin-GK immersion significantly decreased the expression levels of pro-inflammatory factors, including *IL-17*, *p105*, *NLRP3*, *Rel*, and *STAT5*, which are known inflammation markers in echinoderms and are highly expressed during SUS infection [42].

Gut microbiota is a complex and ever-changing ecosystem that is typically maintained in a state of equilibrium. However, the invasion of

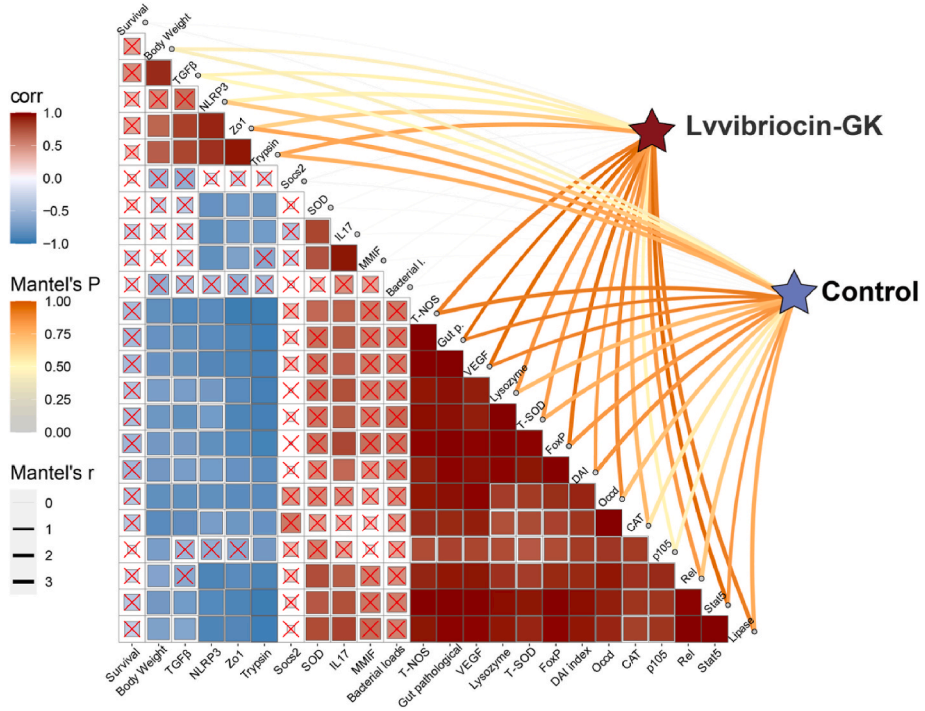


**Fig. 8.** The mantel test between the microbial communities and characterizing host immunity factors in Lvvibriocin-GK immersion treatment and control *A. japonicus*.

pathogens can disrupt this balance, leading to disturbances in both structure and function [43]. Given that SUS is associated with gut microbiota dysbiosis, we further evaluated the effect of Lvvibriocin-GK immersion therapy on the gut microbiota of *A. japonicus* [44]. These findings indicated that Lvvibriocin-GK immersion treatment notably enhanced the diversity of the gut microbiota following SUS infection and increased the abundance of unique ASVs. Previous studies have shown that some *Rhodobacteraceae* family can degrade organic compounds like urea and certain toxins, while also establishing mutualistic relationships with a range of plants and animals [45]. *Bacillus* species have been used as probiotics in *A. japonicus* and other aquaculture species [46]. *S. liquefaciens* contains chitinase and cellulase, which can dissolve bacterial cell walls and inhibit pathogen growth [47]. In contrast, bacteria from *Acinetobacter* are often highly virulent and exhibit broad resistance, potentially leading to enteric diseases in hosts [48], and *B. vulgatus*, a marker for ulcerative colitis, can exacerbate enteric diseases [49]. Consistent with this, our analyses using random forest, indval, and Lefse methods identified differential microbiota associated with Lvvibriocin-GK immersion therapy. Lvvibriocin-GK immersion treatment increased the abundance of potential probiotics, such as *Rhodobacteraceae*, *Bacillus*, and *S. liquefaciens*. In contrast, the control group was marked by a notable increase in opportunistic pathogens such as *Acinetobacter* and *B. vulgatus* (Fig. 6). Network analysis provided a comprehensive view of the composition, aggregation, and interactions within the gut microbiota, revealing dynamic differences between the control and Lvvibriocin-GK immersion treatment groups post-SUS infection [28]. Our results showed that the Lvvibriocin-GK group had a more complex interaction network than the control, which promoted network stability and improved microbiota adaptability, aiding faster recovery after treatment. Additionally, Lvvibriocin-GK immersion therapy enriched the gut microbiota with immune-related functions (Fig. S6), particularly in pathways such as complement and coagulation cascades, clavulanic acid biosynthesis, VEGF signaling pathway, NF-kappa B signaling pathway. This may contribute to enhancing the immune function of *A. japonicus*. Interestingly, not all factors affecting Lvvibriocin-GK immersion treatment of SUS were strongly correlated with gut microbiota. This may be due to the multiple nature of AMP Lvvibriocin-GK's effects: on one hand, it acts as an antimicrobial agent on *A. japonicus*, reducing the bacterial load of pathogens. On the other hand, it has more complex effects in vivo, potentially functioning as an innate immune factor that activates immune-related signaling pathways or enhances enzyme activity [11], and also modulate the gut microbiota to repair the gut barrier function. Furthermore, it might regulate host immune pathways or key signaling factors, such as the NF-kB signaling pathway and key factors like Stat, thereby improving the overall immune response of *A. japonicus* and reducing inflammation [37,50].

Based on the multiple beneficial effects observed with Lvvibriocin-GK treatment and previous findings, we hypothesize that one potential mechanism by which Lvvibriocin-GK alleviates SUS is as follows. Specifically, it may enrich potential beneficial bacteria while suppressing potential pathogens, thereby enhancing intestinal barrier integrity and promoting recovery. Studies have shown that some probiotic species such as *Clostridium* can restore gut barrier function impaired by *Vibrio splendidus* through short-chain fatty acid (SCFA)-mediated upregulation of tight junction proteins like *Zo-1* and *Occludin* [37]. In *A. japonicus*, SCFA levels are significantly reduced following SUS infection, which may contribute to mucus thinning and mucosal barrier damage [38]. As key energy sources for intestinal epithelial cells, SCFAs are crucial for maintaining barrier homeostasis [37]. Notably, members of the genus *Bacillus* are recognized as common SCFA-producing bacteria [51]. Our results show that Lvvibriocin-GK treatment significantly increases the relative abundance of *Bacillus* and other potential beneficial bacteria. Therefore, we propose that Lvvibriocin-GK may exert its therapeutic effects at least in part by enriching SCFA-producing probiotics such as *Bacillus*. This enrichment may promote SCFA production, support the restoration of the mucus barrier, and improve intestinal barrier integrity. This gut microbiota-mediated pathway may represent a potential mechanism underlying the recovery of SUS-affected *A. japonicus*.

Many existing green aquaculture treatments differ from traditional antibiotics, such as probiotics, which are often used as feed additives or water quality enhancers. These treatments typically require long-term use, have a delayed onset of effects, and are primarily aimed at

boosting immunity to prevent SUS [46,52]. In contrast, the AMP Lvvibriocin-GK, studied here, focuses on treating *A. japonicus* with severe SUS and demonstrates promising application potential. However, while Lvvibriocin-GK, as a immersion treatment, effectively alleviates SUS symptoms, promotes epithelial ulcer healing, improves gut microbiota composition, enhances host immunity, and repairs the gut barrier. Additional studies are required to assess the duration of its antimicrobial activity, including the fully decompose time in aquatic environments, the persistence of its effects, its impact on subsequent growth and feeding. In addition, our future investigations will focus on elucidating the antimicrobial mechanisms of Lvvibriocin-GK against *Vibrio* species, its influence on the skin microbiota of *A. japonicus*, and its specific mechanisms of action.

In summary, we present a potential alternative to antibiotic treatment for *A. japonicus* SUS through the use of AMP Lvvibriocin-GK immersion treatment. Under severe SUS conditions, Lvvibriocin-GK immersion treatment significantly improves *A. japonicus* health. This improvement is primarily attributed to the bactericidal activity of the Lvvibriocin-GK against *V. harveyi*, the upregulation of *Zo-1* and *Occludin* expression, which promotes gut barrier repair, as well as enhanced enzyme activities such as trypsin and lysozyme and reduced expression of inflammation-related genes like *IL17* and *p105*. Additionally, the increase in beneficial probiotic abundance, reduction in pathogenic microorganisms, stabilization of the microbiota community, and overall enhancement of immune function may contribute to the efficacy of AMP Lvvibriocin-GK immersion in treating SUS. This study provides an innovative approach to treating severe SUS symptoms and offers new perspectives for developing antibiotic alternatives in aquaculture, providing novel methods and insights for effective SUS management and the healthy development of aquaculture practices.

## CRediT authorship contribution statement

Hang Sun: Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Funding acquisiton. Hui Peng: Methodology, Writing - Original Draft, Writing - Review & Editing, Funding acquisiton. Xiao Hong: Methodology, Investigation, Formal analysis. Fangyi Chen: Writing - Review & Editing, Funding acquisiton. Wenbin Zheng: Methodology, Investigation, Formal analysis. Yuqiao Gao: Methodology, Investigation. Yujun Xu: Investigation. Hua Hao: Investigation. Ke-Jian Wang: Conceptualization, Resource, Supervision, Funding acquisition, Writing-Review&Editing. All authors reviewed the results and approved the final version of the manuscript.

## Ethical approval

The animal study was evaluated and authorized by the Laboratory Animal Management and Ethics Committee of Xiamen University (XMULAC20240211).

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## Declaration of competing interest

The authors declare that this research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2025.110494.

## Data availability

The 16S rRNA gene datasets produced in this study can be accessed in the NCBI repository under accession number PRJNA1158310.

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