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Identification and Characterization of a Novel GAPDH-Derived Antimicrobial Peptide From Jellyfish

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ABSTRACT

Marine organisms serve as a rich source of bioactive natural compounds, including antimicrobial agents. Jellyfish, which are ancient marine invertebrates with hundreds of millions of years of evolutionary history, have been in continuous contact with a diverse array of pathogenic microorganisms from seawater, which may give rise to a distinctive innate immune system and related defensive molecules. However, it is difficult and inefficient to isolate active ingredients directly from jellyfish for enrichment, though few jellyfish-sourced antimicrobial peptides (AMPs) have been reported. In this study, we utilized transcriptomic big data with bioinformatic tools to dig deeper into potential antimicrobial components in jellyfish, and identified a new AMP JFP-2826 from *Rhopilema esculentum*. The 20-mer peptide exhibited an alpha-helix structure and showed antimicrobial activity against selected bacterial strains; more importantly, JFP-2826 demonstrated good selectivity for marine-specific *Vibrio* including *Vibrio vulnificus*. Sequence analysis of the full-length protein of JFP-2826 revealed that it is derived from the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is probably produced through enzymatic cleavage of the N-terminal fragment. This suggests that GAPDH of jellyfish might have a newly discovered antimicrobial-related function that is conducted by releasing JFP-2826-like cryptic peptides. JFP-2826 can be subjected to further structural modifications and optimizations to potentially become a potent lead peptide for the development of novel antimicrobial drugs treating infections of marine pathogens.

1 | Introduction

As a vast ecosystem on the planet, the oceans are extremely rich in biodiversity. A considerable number of lead compounds have been discovered from marine organisms, including many of them with antibacterial activities [1, 2]. Despite lacking an adaptive immune system, marine invertebrates are able to survive in competitive and complex marine environments, implying the evolution of a unique immune defense mechanism [3]. Many studies have reported antimicrobial peptides (AMPs) isolated from marine organisms, which are endogenously synthesized to resist the invasion of pathogenic microorganisms [4]. They not only have antibacterial activities against a wide range of bacteria [5], fungi and viruses,

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but also possess diverse biological functions such as anti-inflammatory, anticancer [6], immunomodulatory [7], and wound-healing effects [8], which play important roles in the fields of medicine, food processing, aquaculture and animal husbandry.

Jellyfish, members of the phylum Cnidaria, are ancient marine invertebrates that have undergone evolution over hundreds of millions of years. This group encompasses a vast array of species and are found across diverse marine environments. Their body cavities are open and immersed in seawater, with long, thin tentacles that form a large contact area with the marine environment. Prolonged exposure to a variety of pathogens on their body surface may have led to the evolution of a unique innate immune system and the production of antibacterial molecules. However, it is difficult and inefficient to directly isolate and enrich the active ingredients from jellyfish, with limited literature on AMPs derived from jellyfish [9].

Additionally, with the rapid advancement of high-throughput sequencing technology and bioinformatics, scientists have been able to sequence the genomes, transcriptomes, and proteomes of numerous species, including jellyfish. This has facilitated the establishment of multi-omics databases and provided valuable insights into their genetic makeup and biological processes. At the same time, several AMP databases have also been established, including the CAMPR4 and ADP3 databases. These databases provide researchers with valuable reference information [10] for online prediction and other functions. Therefore, it is now feasible to employ big omics data and bioinformatics methods to perform high-throughput screening of AMPs. For example, Rostaminejad Marzieh et al. identified three new potential AMPs from the venom of the scorpion *Chaerilustrico status* using existing proteomics data sets [11].

Rhopilema esculentum is among the most prevalent jellyfish species in China. This marine organism is recognized as both medicinal and edible resources in traditional Chinese medicine. In this study, we used transcriptomic big data and bioinformatics tools to thoroughly explore the potential antimicrobial ingredients in *R. esculentum* and identified a new AMP JFP-2826. We analyzed its structure and antimicrobial activities against *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Vibrio vulnificus (V. vulnificus)*, and found that it had good selectivity against marine-specific *Vibrio* strains. Sequence analysis of the full-length protein revealed that JFP-2826 originated from the housekeeping gene GAPDH, indicating that GAPDH of jellyfish might possess previously unreported antibacterial-related functions.

2 | Materials and Methods

2.1 | Screening of Jellyfish Transcriptomes for Potential AMPs

The transcriptome datasets of the jellyfish *R. esculentum* were downloaded from the public database NCBI (https://www.ncbi.nlm.nih.gov/nuccore/GFYS00000000.1); We translated the transcribed sequences into amino acid sequences to predict the likelihood of AMPs in the AMP database CAMPR4. We predicted the sequences with a probability greater than 0.9 using a window size of 20aa. Next, we evaluated the peptide's antibacterial effectiveness against *E. coli, S. aureus*, and *V. vulnificus* was predicted in the DBAASP database using the method Strain-specific antibacterial prediction based on machine learning (ML) approaches and data on peptide sequences and bacterial genomes. The *V. vulnificus* genome ID used was (SAMN05196200). The average scores of the active sequence calculations were calculated and ranked, and the top 10 peptides were selected for solid-phase synthesis. All peptides used in this study are solid-phase synthesized with a purity of 95%, sourced from ChinaPeptides Co., Ltd. in Shanghai, China.

2.2 | Molecular Structure Analysis

The structure of JFP-2826 was predicted by I-TASSER and Phyre2 and observed by PyMOL. The secondary structure of the AMP JFP-2826 was assessed through circular dichroism (CD) spectroscopy. The spectra were analyzed in H_2O and with sodium dodecyl sulphate (SDS) to mimic the peptide's structure in aqueous conditions and under the influence of the bacterial membrane's negative charge. HeliQuest constructed the helix wheel structures.

2.3 | Bacteria Strains Preparation and Growth Conditions

E. coli (ATCC 25922), *S. aureus* (ATCC 25923), and *V. vulnificus* (ATCC 27562) were obtained from Fuxiang Biotechnology (Shanghai, China). These strains were cultured on Luria-Bertani (LB, Sangon Biotech, Shanghai, China) agar plates to activate the bacteria, followed by overnight incubation at 37°C in an incubator. Streaking was performed repeatedly on nutrient-rich LB agar plates to isolate pure bacterial colonies. The bacterial strains were then grown in LB broth, composed of 10 g/L peptone, 10 g/L NaCl, and 5 g/L yeast extract. They were then incubated at 200 rpm and 37°C for 12 h.

2.4 | Measurement of Antibacterial Activity

E. coli, S. aureus and *V. vulnificus* cells were cultured in cation-adjusted Miller-Hinton broth (CA-MHB, Haibo Biotechnology, Shandong, China) at 37°C. The minimum inhibitory concentration (MIC) of the AMP was assessed using the microdilution technique [12]. Briefly, two-fold serial dilutions of AMP ranging from 0.87 to 445.88 μM were introduced into the CA-MHB medium that contained a bacterial culture in the logarithmic growth phase (5×10^5 CFU/mL). Ampicillin, a standard antibiotic, served as the positive control in the experiment, and the well without AMP was used as a control. The final volume of each well was 100 μL. Incubate the samples at 37°C for 16–24 h. After incubation, measure the absorbance at 600 nm using a full-wavelength microplate reader, Multiskan 1550 (Thermo, USA). Plot the peptide concentration on the x-axis and the corresponding absorbance values on the y-axis to create a bar chart. The MIC is identified as the lowest concentration of AMP that effectively inhibits bacterial growth.

2.5 | Time-Kill Kinetic Assay

The bactericidal kinetics were assessed following the Wei method [13], with slight adjustments. Activated cultures of *E. coli, S. aureus*, and *V. vulnificus* were incubated at 37°C with shaking at 200 rpm until they reached the logarithmic growth phase. The bacterial concentrations were then adjusted to 1×10^6 CFU/mL using LB medium. AMP solution was added to make the final concentrations of 1×MIC, 5×MIC, and 10×MIC, respectively, mixed well, and incubated at 37°C for 0, 5, 10, 30, 60, 180, and 240 min. Each time, a 10 μL sample was taken and diluted with fresh broth to an appropriate level. Then, 100 μL of the diluted solution was plated on LB agar plates for inoculation. The bacterial fluid group without AMP was used as a negative control, and the bacterial fluid group with ampicillin was used as a positive control. Following a 24-h incubation at 37°C, a time-killing kinetic curve was generated, plotting time on the x-axis and the number of colonies on the y-axis.

2.6 | Biofilm Inhibition and Eradication Activities

The inhibition ability of JFP-2826 against biofilm formation was determined using crystal violet staining [14]. One hundred eighty microliters of 1×10^5 CFU/mL *E. coli, S. aureus*, and *V. vulnificus* were added to 96-well plates containing 20 μL of a serial 2-fold dilution of JFP-2826, respectively. The bacterial culture group without AMP was set as the control group, and the LB medium group was set as the blank group. After 24 h of incubation, the samples were washed using a sterile phosphate-buffered saline (PBS) solution to remove bacteria floating on the surface. Next, the cultures were exposed to 99% methanol for 15 min for fixation. Subsequently, the methanol was removed, and the 96-well plates were allowed to air dry naturally. Afterwards, 200 μL of crystal violet staining solution at a concentration of 0.1% was added to each well and allowed to stand for 15 min to complete the staining process. The excess stains were then removed by gently rinsing with distilled water. Ultimately, the stained samples were immersed in 95% ethanol to dissolve the dye, which allowed for measuring the absorbance at 600 nm.

To test the destruction of formed biofilm by JFP-2826, 200 μL of bacterial cells (1×10^5 CFU/mL) were added to 96-well plates and incubated at 37°C for 24 h for biofilm formation. After PBS washing, 200 μL of serial dilutions of JFP-2826 prepared in fresh LB medium were added to the wells containing biofilms, and 200 μL of culture medium was added to the control and blank wells. Plates were cultured at 37°C for 24 h and then stained with crystal violet for absorbance measurement as described above. The formula for calculating the percentage of biofilm formation was $(OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100\%$.

2.7 | Prediction of Complete Protein Sequences and Their Cleavage Sites

We found the full-length sequence of JFP-2826 in the downloaded transcriptome and then translated the nucleotide sequence into an amino acid sequence in Expasy-Translate tool and obtained the full-length protein sequence of JFP-2826. The structure of the full-length protein of JFP-2826 was predicted by Phyre2 and observed by PyMOL. We then predicted the cleavage site of the full-length JFP-2826 protein using EXPASY PeptideCutter software.

2.8 | Statistical Analysis

Analyses were performed using the GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). All data were presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was conducted to evaluate differences between the two groups, followed by Tukey's post hoc test. p-value < 0.05 was considered significant.

3 | Results

3.1 | Identification of Potential AMPs From Jellyfish Transcriptomes

We translated the transcribed sequences into amino acid sequences to predict the likelihood of AMPs in the AMP database CAMPR4 and predicted the sequences with a probability greater than 0.9 using a window size of 20aa. The antimicrobial activity of peptides was predicted using a strain-specific antimicrobial prediction method based on peptide sequences and bacterial genomic data from the ML method and the DBAASP database. The average scores of the active sequence calculations were calculated and ranked, and the top 10 peptides were selected for solid-phase synthesis. Supplementary Table S1 shows the MIC of the synthesized peptide fragments against common pathogens and marine pathogens determined by microbial broth dilution assay; a candidate AMP was identified and designated as JFP-2826 (FGRIGRLVLRASLKTKKVTV).

3.2 | Structure Analysis of JFP-2826

The antimicrobial activity of JFP-2826 against *E. coli, S. aureus* and *V. vulnificus* was predicted online using DBAASP as shown in Figure 1A; JFP-2826 consists of 20 amino acid residues. The structure of JFP-2826 was predicted by Phyre2 and observed by PyMOL, as shown in Figure 1B. Results of CD spectroscopic analysis of JFP-2826 showed random curls in H_2O with negative minima between 180 and 200 nm, which were used to model aqueous environments. However, a bacterial membrane simulation environment showed an α-helical structure with a negative minimum between 202 and 214 nm (Figure 1C). JFP-2826 has an opposite content of α-helix and β-folded structures in H_2O versus in 30 mM SDS solution, indicating that the peptide induces α-helix generation in 30 mM SDS to undergo structural alterations favorable for survival in the membrane environment. The helix wheel structures were constructed by HeliQuest, as shown in Figure 1D. Lysine and arginine residues of JFP-2826 contributed to the positive charge (area in blue) on the peptide surface.

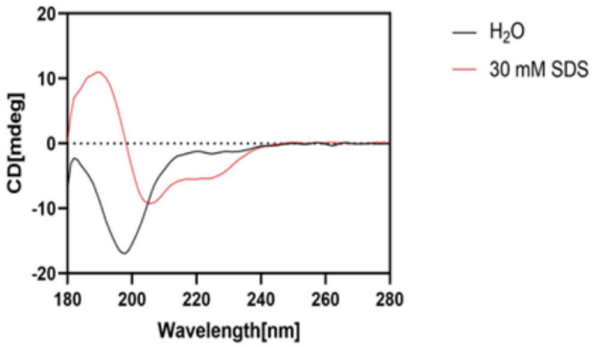
3.3 | Antibacterial Activity of JFP-2826

The MIC results showed that the MIC of JFP-2826 was about 111.47 μM against *E. coli* and *S. aureus*. In comparison, the

A

ID	Strain Type	Class	Predictive value
JFP-2826	*Escherichia coli ATCC 25922*	Active	0.993
JFP-2826	*Staphylococcus aureus ATCC 25923*	Active	0.917
JFP-2826	*Vibrio vulnificus*	Active	0.997

C



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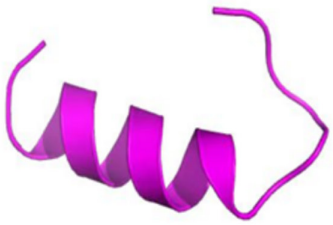

JFP-2826 FGRIGRLVLRASLKTKKVTV

D

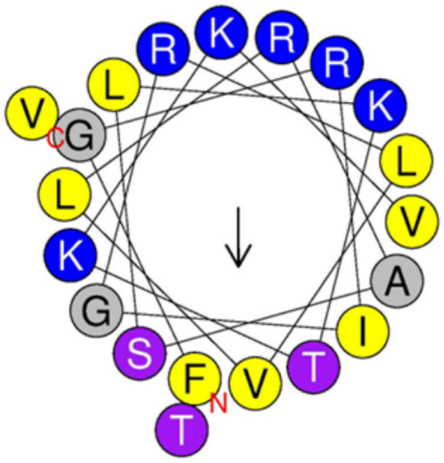

FIGURE 1 | Characterization and structure analysis of JFP-2826. (A) Values of JFP-2826 for the prediction of antimicrobial activity in *E. coli, S. aureus* and *Vibrio vulnificus*. (B) 3D conformation of JFP-2826 using Phyre2 and PyMOL. (C) Secondary protein structural analysis of JFP-2826. The peptide concentration was fixed at 50 μM. (D) Helical wheel diagram of JFP-2826.

MIC of JFP-2826 against *V. vulnificus* was about 55.73 μM, as shown in Figure 2, which suggested that JFP-2826 have good antibacterial activity and probable selectivity against *V. vulnificus*. Therefore, we conducted additional antibacterial experiments of JFP-2826 against another four strains including *Pseudomonas aeruginosa* and three marine *Vibrio* bacteria (Table 1). We found that its antibacterial activities against marine *Vibrio* strains (MICs = 13.93 ~ 55.73 μM) were higher than those against conventional strains (MICs = 111.47 ~ 445.88 μM), which implied that the antimicrobial potential of JFP-2826 might be selective for marine-specific Vibrio.

3.4 | Antimicrobial Kinetics

Examine the antimicrobial activity of JFP-2826 by determining the killing time of JFP-2826 against *E. coli, S. aureus* and *V. vulnificus*. As shown in Figure 3, when the bacterial concentration of JFP-2826 was 5 × MIC, the number of colonies of *E. coli* could be killed within 240 min, and the number of colonies of *V. vulnificus* could be killed within 60 min. However, it only had an inhibitory effect on *S. aureus*. These results indicate that JFP-2826 has time- and dose-dependent lethal effects on *E. coli* and *V. vulnificus*.

3.5 | Biofilm Inhibition and Eradication Activities

A biofilm is a structured community of bacteria encased in an extracellular polymer matrix that adheres to surfaces. Bacterial biofilms may become resistant to antibiotics, making them challenging to treat [15]. *E. coli, S. aureus* and *V. vulnificus* were used to determine whether JFP-2826 inhibits biofilm formation and disrupts formed biofilms. Figure 4A,B showed the inhibition of biofilm formation of *E. coli, S. aureus* and *V. vulnificus* by different concentrations of ampicillin and JFP-2826, respectively. Figure 4C,D showed the disruptive effects of different concentrations of ampicillin and JFP-2826 on formed biofilms of *E. coli, S. aureus* and *V. vulnificus*, respectively. The results showed that both ampicillin and JFP-2826 significantly inhibited the formation of *E. coli, S. aureus* and *V. vulnificus* biofilms, disrupting the formed biofilm activity at MIC concentrations.

3.6 | Prediction of the Origin and Production of JFP-2826

To explore the source of JFP-2826, we analyzed its full-length nucleotide and protein sequence, GFYS01022826.1, which was found to be derived from the housekeeping gene

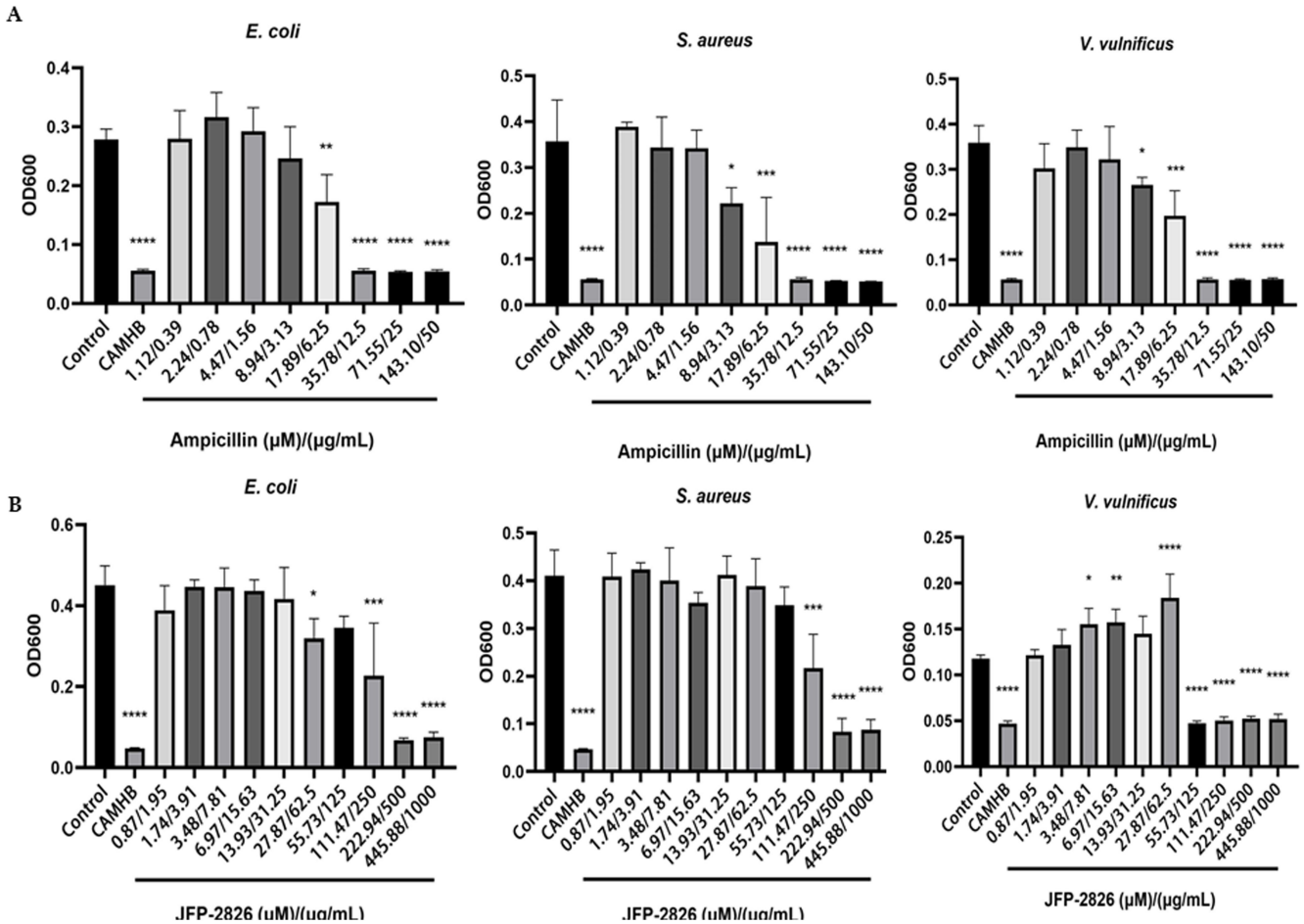

FIGURE 2 | MIC of (A) Ampicillin and (B) JFP-2826 against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *V. vulnificus* (ATCC 27562), $n = 3$. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$ versus control.

TABLE 1 | MIC of JFP-2826 against seven bacterial strains.

Strain type	MIC (μM)/(μg/mL)
E. coli ATCC25922	111.47/250
S. aureus ATCC25923	111.47/250
V. vulnificus ATCC27562	55.73/125
V. parahaemolyticus ATCC17802	27.87/62.5
V. alginolyticus ATCC27853	13.93/31.25
V. harveyi ATCC BAA-1117	13.93/31.25
P. aeruginosa ATCC27853	445.88/1000

GAPDH. The source amino acid sequence of the AMP is QVGINGFGRIGRLVLRASLKTKKVTVVAVNDPFLPIDKMIYLFTHDTVHGNCGVPVSKDDNTLVVDGNEITVYAERDPTKIPWGTSKVDVVVESTGIFTDLDKAS, wherein the JFP-2826 fragment is located at 7–26 aa (Figure 5A). Using the Expasy PeptideCutter tool, we recognized multiple cleavage sites of Asp-N endopeptidase in the full-length protein. When the first site between the 30th and 31st amino acid residue is cleaved, the resulting fragment (aa 1–30) approximates JFP-2826 (Figure 5B). As shown in Figure 5C, JFP-2826 constitutes the N-terminal helix domain in the tertiary structure of the complete protein.

To verify if JFP-2826 could be a mature peptide released from the jellyfish GAPDH, we performed recombinant protein expression, purification, and enzyme digestion of GFYS01022826.1 followed by mass spectrometry (MS) analysis (Supplementary Material, Figures S1–S7). The purified protein was digested by Asp-N endopeptidase and chymotrypsin, respectively. The peptide identification results of enzyme-digested product by MS showed that Asp-N endopeptidase hydrolyzed the protein at the N-terminal of the first aspartic acid (D31), indicating that the N-terminal fragment containing JFP-2626 could be actually released from the full-length protein. Moreover, this fragment could be further cleaved by chymotrypsin or other enzymes expressed in jellyfish to produce the 20-mer peptide JFP-2826.

4 | Discussion

Our preliminary studies suggest that the antimicrobial activity of JFP-2826 against marine Vibrio strains is superior to that against conventional strains, which may be related to the adaptive evolution of jellyfish. Jellyfish live in the marine environment and are in permanent contact with marine pathogenic microorganisms. Since *V. vulnificus* is a common marine-specific Vibrio bacterium that can cause severe infection, sepsis and death in the host, the innate immune system of jellyfish might develop

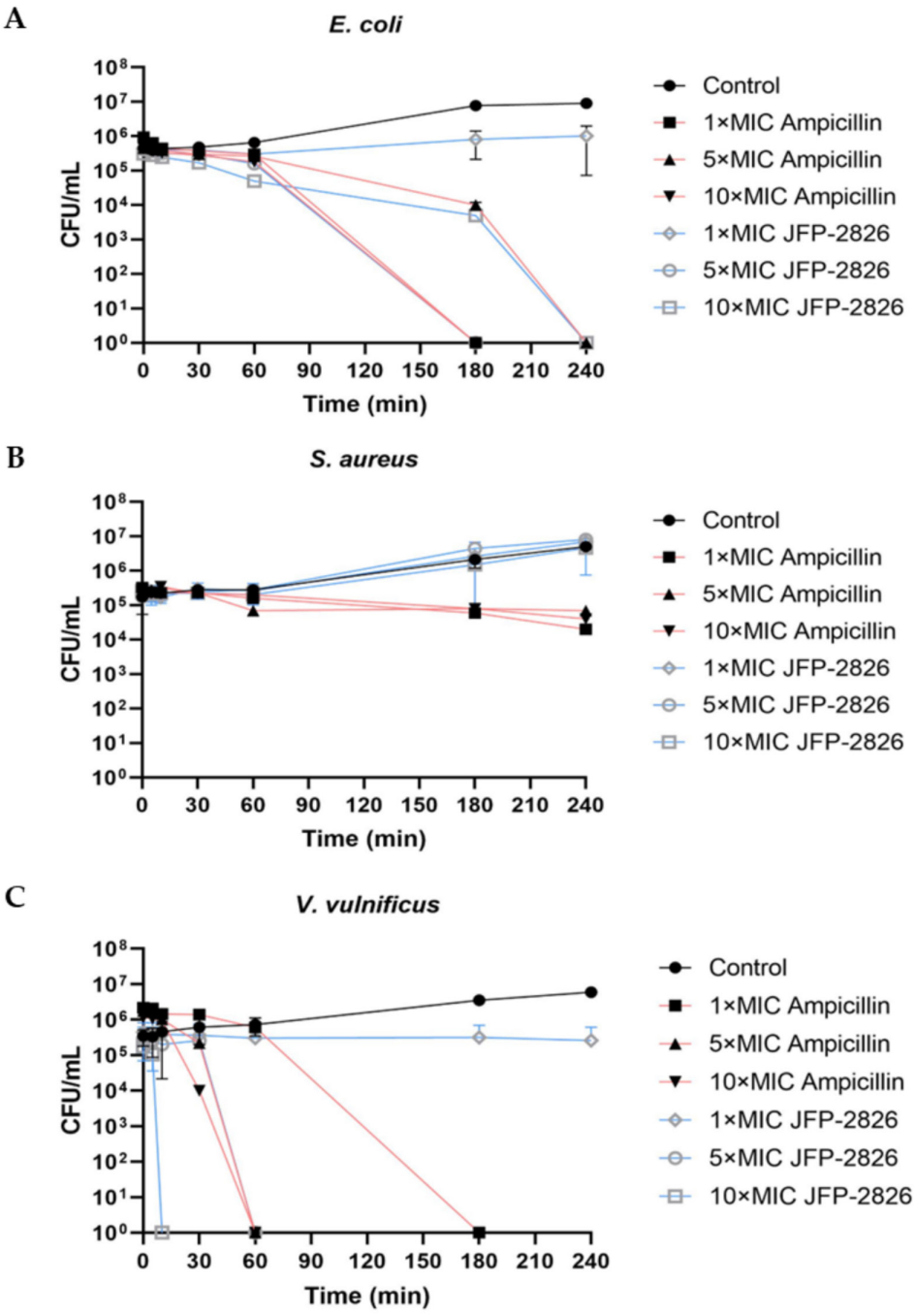

FIGURE 3 | Antimicrobial kinetics of JFP-2826 and ampicillin. (A) *E. coli* (ATCC 25922), (B) *S. aureus* (ATCC 25923) and (C) *V. vulnificus* (ATCC 27562) were incubated with JFP-2826 and ampicillin at 1×MIC, 5×MIC and 10×MIC values.

a specific defensive response as well as antimicrobial molecules to resist its invasion, which is supposed to partially explain why JFP-2826 had better antimicrobial activity against *V. vulnificus*. In addition, JFP-2826 killed *V. vulnificus* colonies within 60 min at a concentration of 5×MIC, and this rapid killing effect was also less likely to lead to bacterial resistance. As some broad-spectrum antimicrobials in the clinic lack specificity and tend to cause unwanted cytotoxicity during systemic administration, the good selectivity and bactericidal efficiency of JFP-2826 towards marine *Vibrio* strains may portend a high safety profile in treating infections with marine pathogens [16].

In addition to these "conventional" cationic antimicrobial peptides (CAMPs), there are an as-yet undetermined number of CAMP-like cryptides derived from defense proteins such as lysozyme, lactoferrin, ribonuclease, or complement proteins, as well as proteins that are not directly involved in defense such as coagulation factors, extracellular matrix components, or even histones, extracellular matrix components and even histones [17]. In multicellular eukaryotes, producing bioactive peptides exerting blood pressure regulation, regulation of proteolytic enzymes, analgesic functions, etc., from proteolytic hydrolysis of larger proteins is a common strategy [18–20]. Such

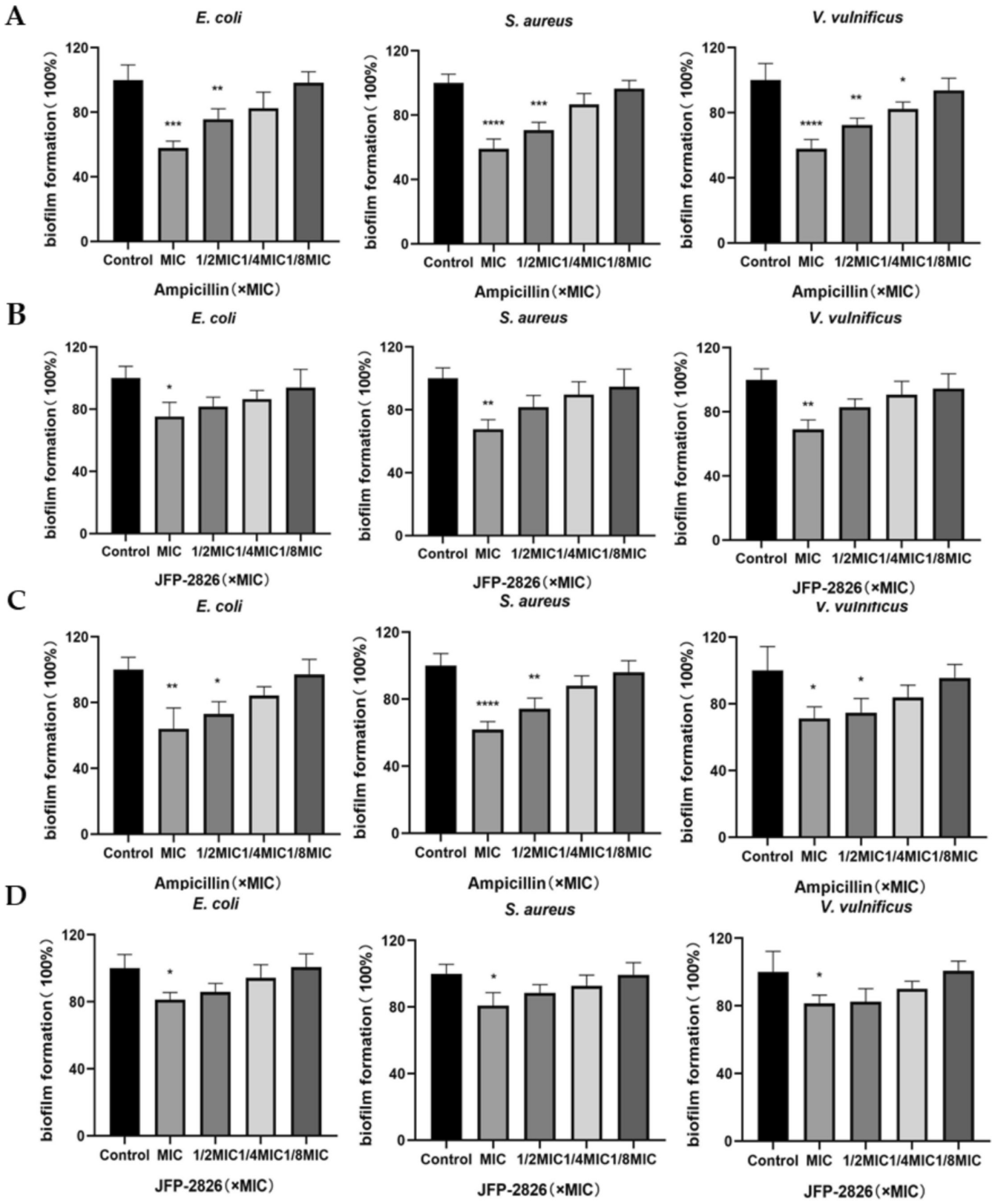

FIGURE 4 | Biofilm inhibition and eradication activities of JFP-2826 and ampicillin. *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *V. vulnificus* (ATCC 27562) were treated with different concentrations of ampicillin (A) and JFP-2826 (B) to measure the inhibition of biofilm formation. *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *V. vulnificus* (ATCC 27562) were treated with different concentrations of ampicillin (C) and JFP-2826 (D) to measure the eradication of biofilm formation, $n = 3$. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$ versus control.

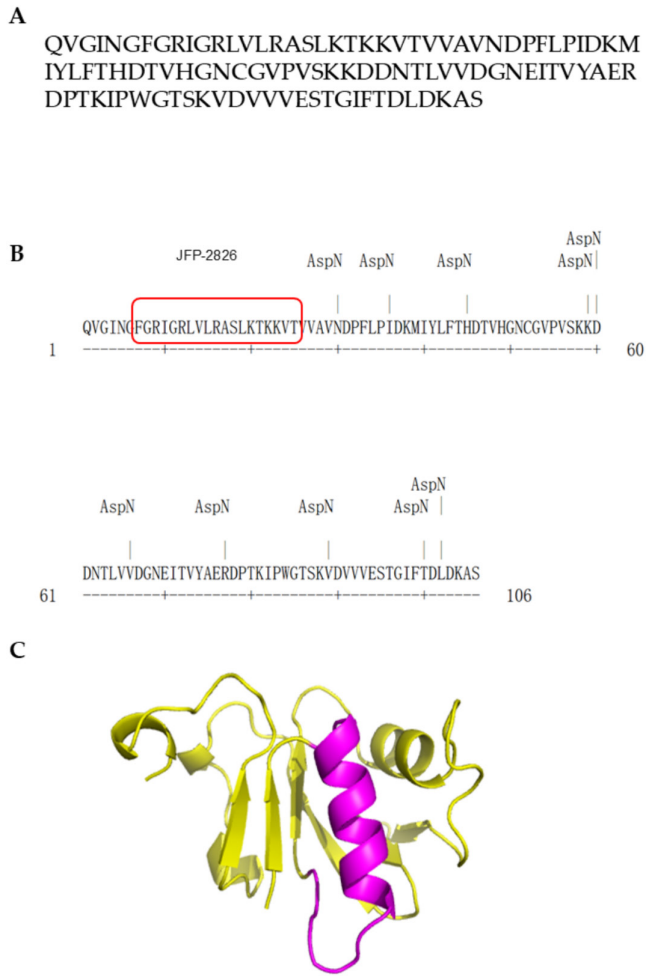

FIGURE 5 | Prediction of complete protein sequences and cleavage sites of JFP-2826. (A) Sequence of the full-length protein of JFP-2826. (B) Predicted cleavage sites of the full-length protein. JFP-2826 sequence was shown in red frame. (C) 3D structure model of the full-length protein using Phyre2 and PyMOL.

bioactive cryptic peptides and proteins are called “cryptide” and “cryptein”, respectively [21]. Most known cryptic CAMPs were identified either serendipitously or through their similarity to previously recognized cryptides. Pane has developed a computer simulation tool that identifies and localizes cryptic peptides with great precision analyzes the internal structure of long CAMPs [22]. This is also an approach to designing and developing novel therapeutic peptide analogs or mimetics based on host defense peptides [23]. Nevertheless, in the case of known antimicrobial cryptides, descriptions of immunomodulatory and other biological activities still need to be included. Except for very few cryptic peptides, such as those derived from lactoferrin [24, 25] and thrombin [26], we do not know when, where and how cryptic peptides are released. This means that our current understanding of innate immunity is sorely lacking.

In this study, we found the AMP JFP-2826 sourced from the housekeeping gene GAPDH of jellyfish. Enzyme digestion and MS results demonstrated that JFP-2826 and related peptide fragments were able to be released from the N-terminal of the source protein through cleavage by Asp-N endopeptidase combined with chymotrypsin and other possible enzymes expressed in jellyfish. The N-terminal sequence from GAPDH was similarly isolated from the epidermis of skipjack tuna and yellowfin tuna, and the N-terminal fragment of this protein has antimicrobial activity against Gram-positive and Gram-negative bacteria [27], and the antifungal efficacy of GAPDH-derived peptides has been demonstrated in many studies [28, 29]. In addition, two genes involved in glycolysis in grouper (gapdh 1 and gapdh 2) are predicted precursors of AMP with high homology to GAPDH-related AMPs in skipjack tuna and yellowfin tuna, respectively [30]. The origin of these AMPs produced from different organisms all point to GAPDH. This suggests that GAPDH is not only an enzyme involved in glycolysis in organisms, but also an important component of the organism's defense system. Since the related homologous peptide fragments longer or shorter than JFP-2826 also have predictive antimicrobial potential, jellyfish GAPDH might have a newly-discovered antimicrobial-related function conducted by releasing JFP-2826-like cryptic peptides. This phenotype is likely to occur under specific growth conditions or cellular environments, such as during pathogen infection. However, this still needs further investigation to confirm its possibility as well as the actual length of released antimicrobial fragments by in vivo experiments in jellyfish.

5 | Conclusions

In this study, we utilized transcriptomic bigdata with bioinformatic tools to dig deeper into potential antimicrobial components in jellyfish, and identified a new AMP JFP-2826 from *R. esculentum*. The 20-mer peptide exhibited an alpha-helix structure and showed antimicrobial activity against selected bacterial strains; more importantly, the antimicrobial potential of JFP-2826 showed selectivity for marine-specific *Vibrio*. Sequence analysis of the full-length protein of JFP-2826 revealed that it was derived from the housekeeping gene GAPDH, which was probably produced through enzymatic cleavage of the N-terminal fragment. These results suggested that GAPDH of jellyfish might have a newly-discovered antimicrobial-related function that was conducted by releasing JFP-2826-like cryptic peptides. In a word, this study identified a new source of antimicrobial agents. We thought that JFP-2826 can be subjected to further structural modifications and optimizations (e.g. increasing the positive charges by cationic amino acid substitution) to potentially become a potent lead peptide for the development of new antimicrobial drugs treating infections of marine pathogens.

Author Contributions

Conceptualization, J.Y., M.W., Q.W., J.L., A.L., and Y.L.; methodology, J.Y. and A.L.; format, J.L. and Q.W.; validation, J.L., Y.L., X.G., and J.W.; resources, Q.L. and M.W; data cu-ration, J.L.; writing – original draft preparation, J. L, A.L., and Y.L.; writing – review and editing, M.W., Q.W., J.Y.; project administration, J.Y.; funding acquisition, J.Y.; J.L., A.L. and Y.L. contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

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Consent

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

Institutional Review Board Statement

Not applicable.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.