Bioinformatics exploration of identified garlic-derived antimicrobial peptides: a food-based approach to quorum sensing inhibition in foodborne pathogens

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Abstract

The growing frequency of antibiotic-resistant microorganisms requires novel antimicrobial methods. Quorum Sensing (QS), a bacterial communication system, is critical for controlling virulence factors and biofilm development, contributing to the pathogenicity of many foodborne bacteria. Garlic is a widely consumed plant with antimicrobial properties and antibacterial capabilities, although its peptide components are poorly unknown. This study evaluated garlic-derived peptides' ability to inhibit QS in foodborne bacteria. Two garlic-derived peptides, including VS-9 and F3-3-c, undergo bioinformatics research to determine their structural features, bioactivity, physicochemical parameters, and potential interactions with target modeled proteins of LasR QS from *Pseudomonas aeruginosa*, Biofilm-associated surface protein (Baps) from *Staphylococcus aureus*, and sortase A (SrtA) from *Staphylococcus aureus*. VS-9 has the most favorable structure properties, which could be essential for its inhibitory activity against LasR, Baps, and SrtA proteins. We have modeled, characterized, and docked garlic-derived peptides to assess their antimicrobial properties. Even though VS-9 showed more anti QS activity than F3-3-c, more research is needed to fully understand their mechanisms of action and maximize their therapeutic potential.

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Introduction

Quorum Sensing (QS) is a sophisticated cell-to-cell communication process used by bacteria to coordinate group behavior,[1] such as biofilm formation[2,3] and virulence factor production,[4] and is considered a serious challenge for human health. Given QS's critical involvement in bacterial pathogenicity and persistence,[5] addressing this communication system offers a viable avenue for creating novel antimicrobial treatments.

While huge progress has been made in understanding QS under controlled conditions, studying its dynamics in complicated contexts is still critical for the creation of effective QS inhibitors. On the other hand, Sortase enzymes (SrtA) are critical for the survival and virulence of Gram-positive bacteria.[6,7] These enzymes anchor important proteins to the bacterial surface, enabling them to attach to host cells and cause infection. Inhibiting sortases is a promising strategy for developing new antibiotics.[8]

Quorum sensing plays a crucial role in the process of food spoilage by enabling bacteria to act in concert to deteriorate food products.[9] *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. are the most foodborne pathogens that have been shown to develop biofilms on food products[10]. *Pseudomonas aeruginosa* employs a QS system involving the *LasI/R* system to regulate gene expression, including virulence factor production. *P. aeruginosa* uses a type of quorum sensing. The *LasI/R* system is essential to this process. While *LasI* synthesizes signaling molecules, LasR acts as a receptor to trigger specific gene expression. [11] By disrupting this communication, LasR could con-

trol bacterial processes, such as quorum sensing, biofilm formation, and virulence factor production, related to food spoilage and infections. Interestingly, *Staphylococcus aureus*, a Gram-positive bacterium, produces a Biofilm-associated surface protein (Baps), playing an important role in biofilm formation and bacterial adhesion.[12]

Antimicrobial Peptides (AMPs) from natural sources, also known as microbial peptides or peptide antibiotics, act as the first line of defense for many organisms. AMPs are short sequences of amino acids that diverse organisms use as a key defense mechanism.[13,14] These peptides showed broad-spectrum antibacterial activity via cell membrane targeting[15] AMPs have received considerable attention in the context of increasing antibiotic resistance; they are classified according to their origin,[16] and are a promising alternative to standard antibiotics due to their low toxicity and resistance potential.[17]

Garlic is a plant with diverse applications, known for its pungent smell and aroma, which are due to a complex combination of bioactive components such as organosulfur,[18] flavonoids, and saponins.[19] Garlic has shown promise as a source of antibacterial compounds. Importantly, not much is known about the functional roles of garlic proteins, especially peptides. Garlic contains naturally occurring bioactive dipeptides, including gamma-glutamyl-cysteine derivatives.[20,21] These compounds can undergo further modifications to form other bioactive compounds. The specific composition of these peptides varies depending on the garlic source.[22] Although the study of garlic extract and its bioactive compounds have produced insightful results, the study of its peptide constituents has lagged. Therefore, garlic offers a viable study area given the increased interest in finding novel peptides and the need for sustainable supplies.[23,24]

Importantly, the potential of garlic-derived peptides is mostly unknown. Hydrolysis of garlic proteins has led to the identification of antimicrobial peptides, such as Trp-Pro-Thr-Ser-Phe-Thr (WPTSFT), Tyr-Asn-His-Asn-Phe (YNHNF), and Ala-Val-Asp-Arg-Ala-Val (AVDRAV),[25] suggesting the potential for discovering additional peptides with antimicrobial properties. Furthermore, a new peptide, VS-9, with intriguing anticancer characteristics that displayed decreased cytotoxicity against normal human cells was recently identified.[26]

Bioinformatics uses genomic, proteomic, peptidomic, and other biological databases to investigate Protein-Protein Interactions (PPIs). These interactions are crucial for numerous cellular processes, including immune responses, regulation, and enzyme function.[27] Understanding these interaction provides important value for comprehending cellular behavior.[28] The identification of peptide structures that may hinder protein interaction is achieved through bioinformatics.[28]

The limitations of this work are the *in vitro* study methodology, the need for more peptide characterization, the narrow focus on a particular group of peptides obtained from garlic, and the difficulties in converting lab results into marketable products. In this study, from previously identified and characterized antimicrobial peptides derived from fresh and Laba garlic, we use bioinformatics approaches for modeling proteins and peptides to evaluate the effect of these peptides to inhibit QS in foodborne pathogens, to develop novel strategies to combat foodborne illnesses.

Materials and Methods

AMPs modeling, building, and models validation

There is currently no publicly available database specifically dedicated to garlic-derived peptides. Two fresh and Laba garlic-derived peptides, VS-9 and F3-3-c [25,26] respectively (*Table S1*), were selected for further bioinformatic analysis. A web tool (http://avermitilis.ls.kitasato-u.ac.jp/readseq.cgi) was used to convert peptide to FAST-ALL (FASTA) format. We used Chimera X software version 1.8, for peptide building and saved as PDB (Protein data bank) file for further validation. ModRefiner (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/)[29] was used for garlic-derived peptides for refinement. Tachyplesin-1 (TP-1), a well-known antimicrobial peptide was utilized as a positive control.[30] TP-1 was retrieved from UniProt Data Bank (https://uniprot.org/). The automated protein structure homology modeling server (https://swissmodel.expassy.com) was used with shared 100 % of identity and 0.74 for global model quality estimation (GMQE). Peptides model were further refined by PEP-FOLD2 (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOL). The best model was selected for further analysis. Structures were therefore evaluated and validated by using Structural Analysis and Verification Server (SAVES) v6.0. The Discovery Studio Visualizer version 21.1.0.20298 and University of California San Francisco (UCSF) Chimera X version 1.8 were used to visualize and analyze the generated model structure.

In silico evaluation of peptide structure

The three-dimensional structures of the peptides were assessed using the Protein Structure Analysis-web server (ProSA) (https://prosa.services.came.sbg.ac.at/prosa.php). This web-based tool analyzes protein and peptide structures and provides a z-score, reflecting the structure's overall quality. Negative z-scores indicate a higher degree of similarity to native protein structures, suggesting a more favorable conformation.[31]

Proteins 3D structure prediction, refinement, and validation

UniProt Data Bank was used for retrieved proteins of LasR quorum sensing from *Pseudomonas aeruginosa*, Biofilm-associated surface protein (Baps) from *Staphylococcus aureus,* and sortase A (SrtA) from *Staphylococcus aureus*. After BLAST (Basic Local Alignment Search Tool) was run, the protein was downloaded as FASTA canonical format. Swiss-model server (www.swissmodel.expassy.org) was used to build and assess the 3D structural proteins. Structures were therefore refined and validated by using GalaxyRefine web tool (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) and SAVES v6.0 (https://saves.mbi.ucla.edu/), respectively. The ChimeraX software was used to visualize and analyze the generated model, and illustrate figures for protein-peptide docking analysis. The quality of all generated 3D models was assessed using the MolProbity tool integrated within the Swiss-model server.

Assessment of the physicochemical parameters of selected peptides

PepCalc online tool (www.PepCalc.com) was used to determine the physicochemical parameters of the three selected peptides.

Peptide antigenicity analysis

Peptide antigenicity was detected by Vaxijen V 2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html),[32] where peptides with antigenicity score more than 0.4 for the bacterial model were considered antigenic.

Garlic-derived peptides bioactivity prediction

We employed the PeptideRanker tool (http://distilldeep.ucd.ie/PeptideRanker) [33] to evaluate a set of garlic-derived peptides for potential bioactivity. This web-based tool utilizes machine learning models to predict a score for each peptide, which reflects its likelihood of possessing a specific biological function.

Peptides toxicity analysis

We employed the ToxinPred web tool to estimate the potential toxicity probabilities of the garlic-derived peptides (https://webs. iiitd.edu.in/raghava/toxinpred).[34] The toxinPred tool is designed for less than 50 amino acids in length peptides. TP-1, exceeding this limit, cannot be directly analyzed by ToxinPred for toxicity prediction.

Protein–peptide docking

Protein-peptide docking of the garlic-derived peptides with the targeted enzyme was performed using the HDOCK server (www.hdock.phy.hust.edu.cn). HDOCK is a web server for protein-protein and protein-DNA/RNA docking based on hybrid strategy.[35] It combines template-based modeling with free docking to predict how molecules interact. It uses biological information and predicts the best fit using a scoring function.[36]

Results

Garlic-derived peptides homology modeling and structural assessment

A search for homology modeling was used to gain further insights into the structure of the three selected peptides. The 3D structures were analyzed, and structural variations were found between the three selected antimicrobial peptides (Figure 1 A, C, E). After refinement, the garlic-derived peptides Root Mean Square Deviation (RMSD) were improved to 1.718 Å and the template modeling score (TM-score) decreased to 0.4755 compared to the initial model. This indicates that the refinement process might have moved the model away from the initial structure. The ProSA-web Z-scores were suggested varying degrees of structural favorability for the three peptides (Figure 1 B, D, F).

Homology modeling and structural characterization of peptides and proteins

The Expasy Swiss-model results suggest a good quality model with a well-folded backbone and minimal clashes (*Table S2*). The percentage of Ramachandran favored outliers indicates that the majority of the peptide's and protein's amino acid residues (backbone dihedral angles) fall within the allowed regions of the Ramachandran plot (Figure 2; upper panels). The initial structure or model 1 seems to have a better MolProbity score, indicating it adheres better to expected protein geometry and chemistry (*Table S3*). RMSD is a value that measures the average difference between the atomic location in the targeted model and reference structure. Lower RMSD values mean a higher similarity. The distribution of amino acid residues within a core region of the Ramachandran plot (Figure 2) lower panels suggested that most of them acquired energetically advantageous backbone conformations.

Analysis of selected AMPs

Table 1 shows an indication of the molecule's size, electrical properties, and behavior in solution. The positive net charge at pH 7 suggested that VS-9 peptide might interact with negatively charged molecules cell membranes or other charged molecules; for F3-3-c and TP-1, it was showed neutral (Zero) and negative electrical charge at neutral pH, respectively. The result suggests good water solubility, indicating the molecule is likely to dissolve well in water.

Analyzing peptides antigenicity

The results of the peptides antigenicity assessment showed that the current potential VS-9 peptide construct is antigenic with an antigenicity score of 0.7552 (Table 1).

Garlic-derived peptides bioactivity prediction

VS-9 has a score of 0.19, which suggests it might have some bioactivity potential. While F3-3-c peptide was showed to have weaker bioactivity prediction, TP-1 was predicted to have strongest bioactivity potential (Table 1).

Toxicity prediction of peptides derived from garlic

Based on the ToxinPred results (Table 1), both VS-9 and F3-3-c are predicted to be non-toxic. The provided properties support this

Table 1. Characterization of garlic-derived peptides: physiochemical properties, antigenicity, bioactivity, and toxicity analysis.

Peptide name	Number of amino acids	Molecular weight g/mol	Extinction coefficient $M^{-1}cm^{-1}$	Iso-electric point	Net charge at pH 7	Estimated solubility	Antigenicity Probable	Antigenicity Prediction	Bioactivity scoring
VS-9	9	1018.28	0	pH 10.1	1.9	Good	0.7552	Antigen	0.188561
F3-C	6	629.71	0	pH 6.71	0	Good	0.1880	Non- Antigen	0.104948
TP-1	77	9348.69	10810	pH 4.88	-2.3	Good	0.9254	Antigen	0.951494

Peptide name	SVM Score	Prediction	Hydrophobicity	Steric hindrance	Side bulk	Hydropathicity	Amphipathicity	Hydrophilicity	Net Hydrogen	Charge	pI
VS-9	-0.70	N/Tox	-0.13	0.59	0.59	0.90	0.68	-0.14	0.89	2.00	9.55
F3-C	-0.91	N/Tox	-0.15	0.65	0.65	0.67	0.41	0.33	0.83	0.00	6.19

pI: isoelectric point; N/Tox: non-toxic; SVM; support vector machine.

prediction, indicating that these peptides likely have favorable interactions with water.

Protein–peptide docking analysis

The protein-protein docking models of anti-QS in *P. aeruginosa* garlic-derived peptides and LasR protein were predicted by the updated version of HDOCK server. As shown by Figure 3 (upper panels), the binding of QS proteins to garlic-derived peptides has occurred differently. Subsequently, the interface interactions between the protein and the coupled peptide were evaluated using Chimera X and Discovery studio software to more elucidate the hydrogen bond-involved interactions and residues. The inhibition of bacterial surface proteins (Baps) by garlic-derived peptides also has

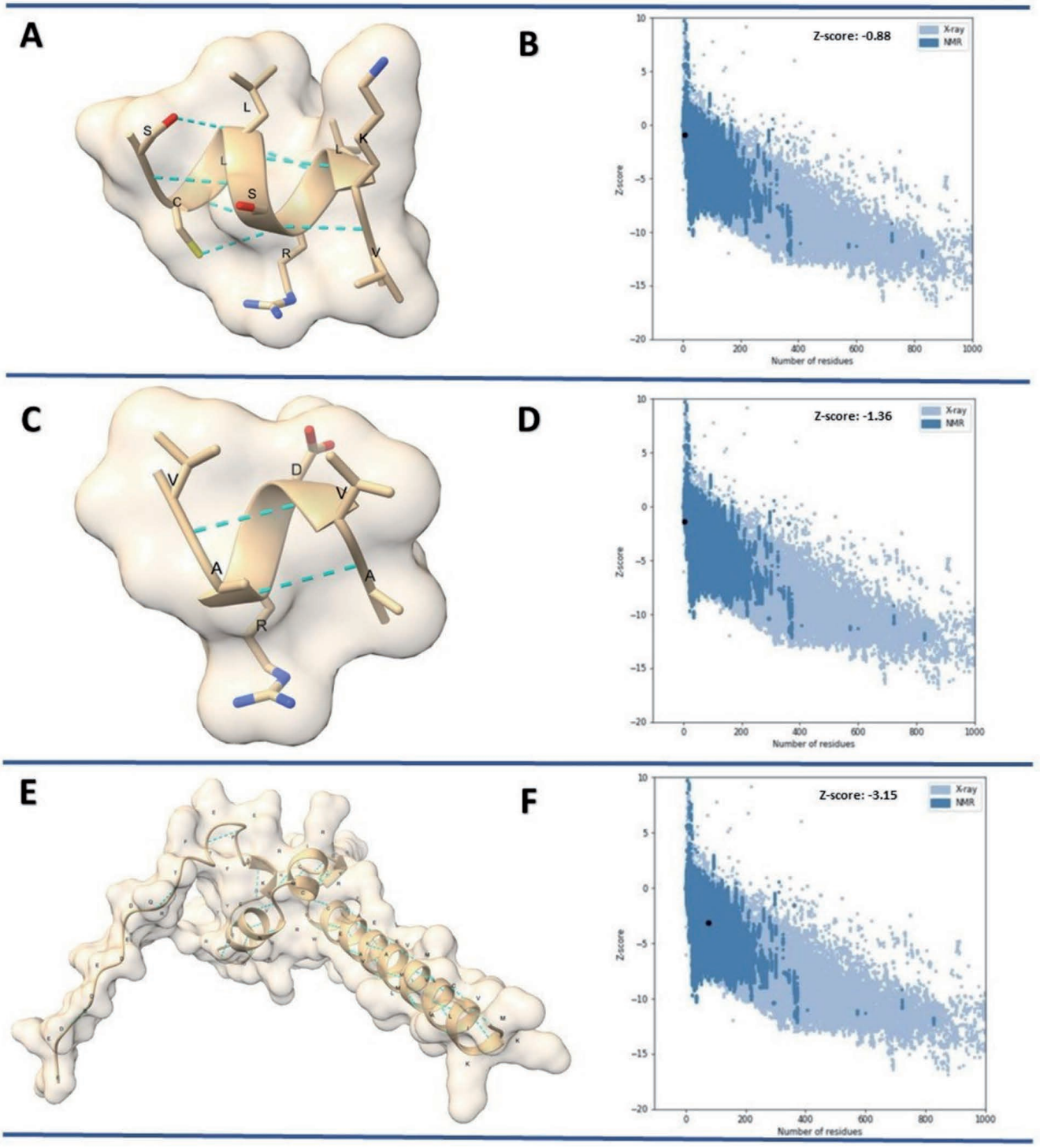

Figure 1. Predicted 3D structures for peptides: (A, C, E) main-chain and side chains are depicted in ribbon and stick representation respectively, H-Bonds are displayed in dashed magnetic colored line; (B, D, F) peptides ProSA-web Z-scores.

been evaluated using molecular docking simulation (Figure 3, middle panels). The result showed this peptide could interact and inhibit biofilm development in *S. aureus*. The interaction between the docked sortase A from the cell wall of *S. aureus* and selected peptides also have been illustrated (Figure 3, lower panels).

Discussion

The increasing prevalence of foodborne diseases, driven by factors such as dietary shifts, and antibiotic resistance,[37] necessitates the development of novel antimicrobial strategies.[38] With its main benefit being for health, garlic is a versatile plant with a long history of medicinal applications. It is also frequently utilized as a food ingredient. However, Laba garlic is a special Chinese food known for its green color and flavor taste. It is a processed garlic food product.[39]

Garlic-derived AMPs most likely work against bacteria by damaging their cell membranes. This is possible through two main mechanisms; the barrel-stave model, in which AMPs embed into the membrane to generate pores, and the carpet model, in which AMPs accumulate on the membrane surface,[25] causing membrane damage and leakage of cellular contents. Gao et al [25] demonstrated three garlic-derived peptides had strong antimicrobial activity against several common foodborne bacteria *in vitro*. Peptide F3-3-c inhibited bacterial growth. This study aligns and supports the rationale for further bioinformatics exploration in this area.

In a 2021 study conducted by Rasaratnam et al.[26] VS-9, an anticancer peptide extracted from fresh garlic, was found. Because of its distinct structure, this peptide can damage the membranes of cancer cells, resulting in cell death. The peptide's amphipathic properties allow it to interact with bacterial proteins and also the lipid components of bacterial cell membranes. The peptide's ability to dual interaction improves the antibacterial agent effectiveness. Importantly, this study represents the first evaluation of VS-9's potential to inhibit quorum sensing in foodborne bacteria using bioinformatics methods (Figure 4).

Chemical preservatives are not the favored method for controlling foodborne pathogens in food and its products.[40] AMPs consider alternative sources of food preservation rather than conventional chemical methods.[41]

Bioactive peptides may offer advantages over conventional small-molecule drugs in some applications. They are smaller than massive protein-based drugs, making them more easily absorbed by the body, and regulate various biological processes [42] with fewer side effects and more immunogenicity. [43,44] VS-9, may have some bioactivity potential while F3-3-c peptide showed an inferior bioactivity prediction.

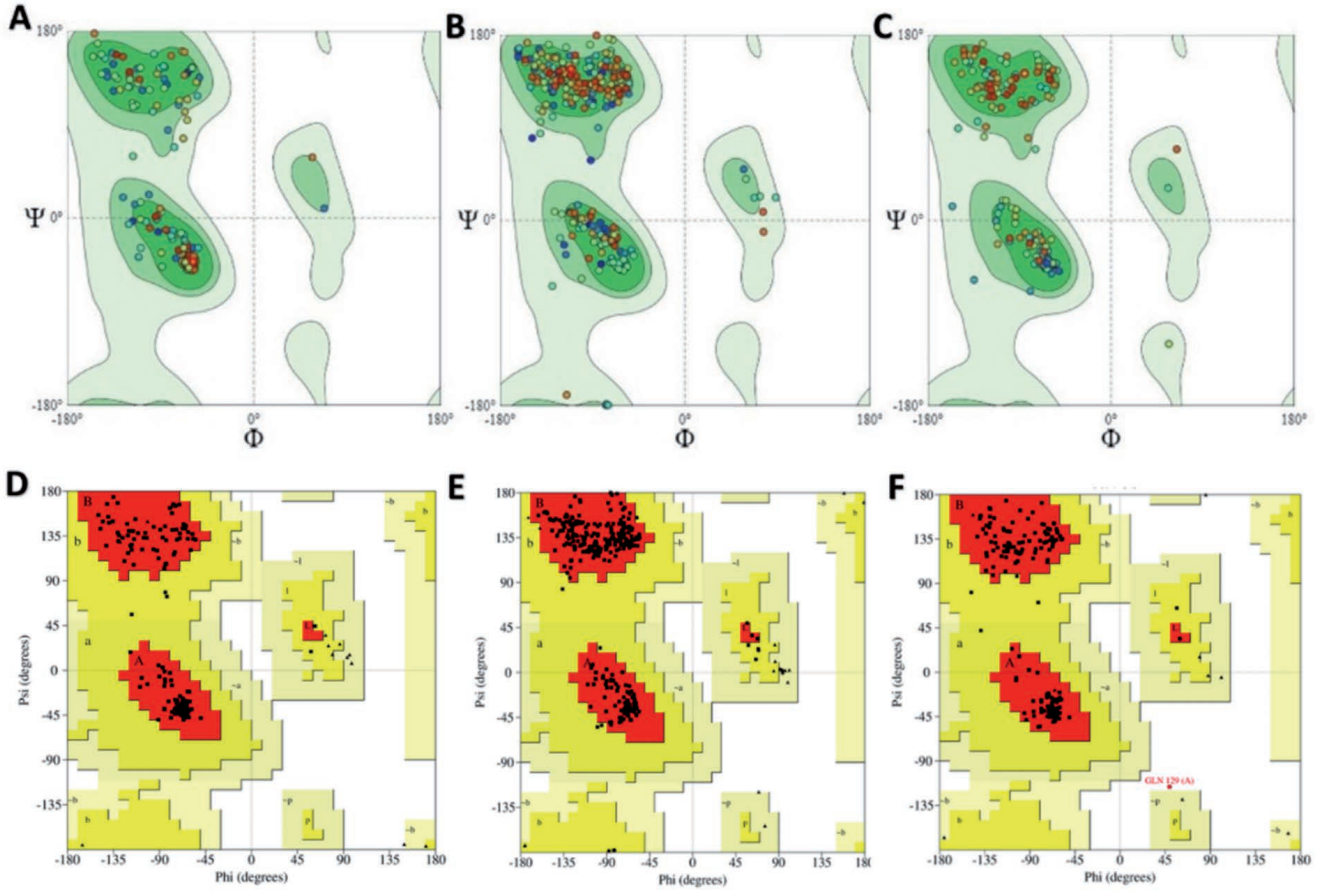

Figure 2. Ramachandran plot analysis of modeled peptides and membrane proteins: Upper panels represent (A) VS-9; (B) F3-3-c; (C) TP-1 peptide. While lower panels represent: (D) LasR quorum sensing from *P. aeruginosa*; (E) Biofilm-associated surface protein (Baps) from *S. aureus*; (F) SrtA from *S. aureus*.

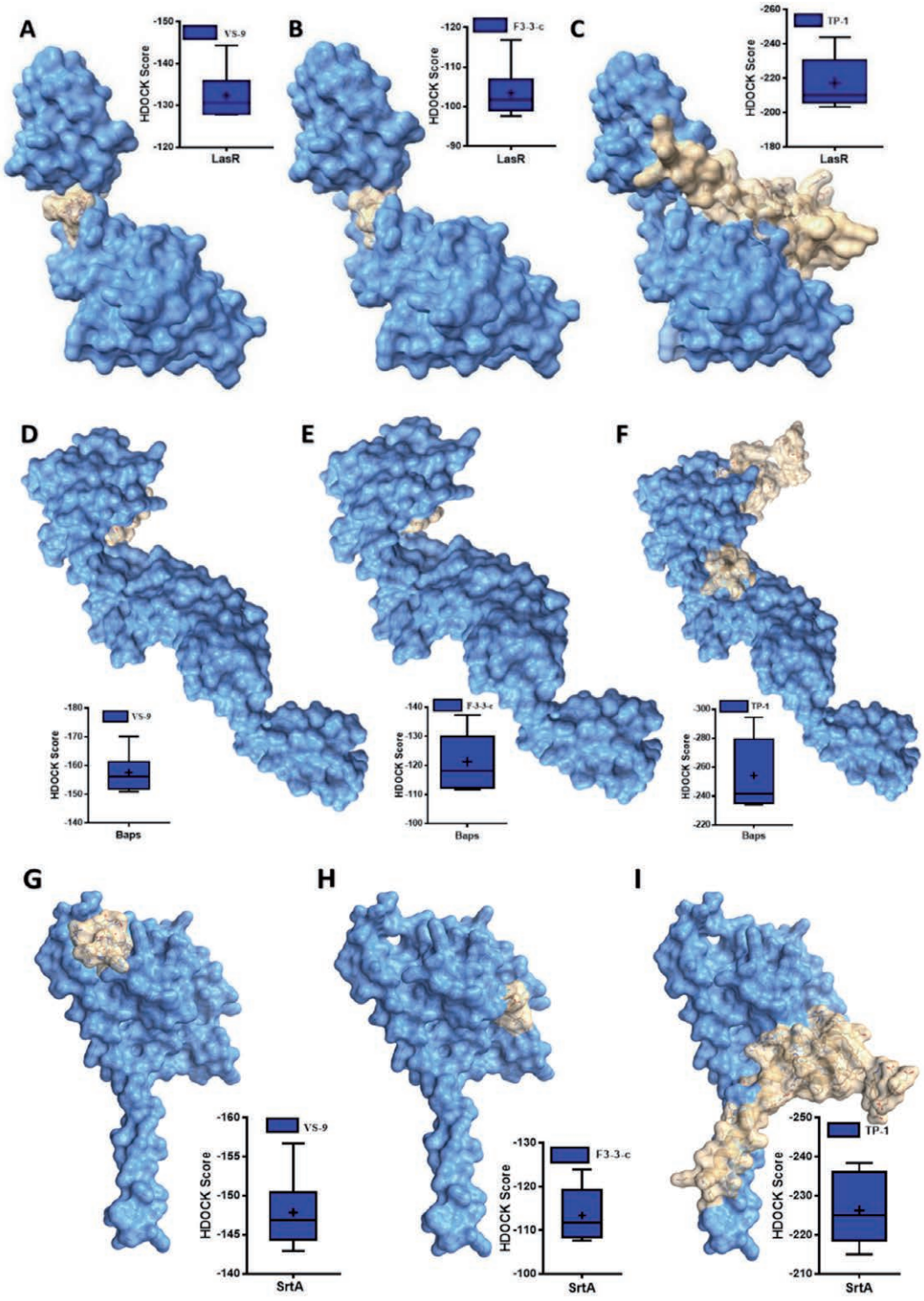

Figure 3. Predicted Protein-Peptide Docking Complexes. Upper panels show modeled proteins of LasR quorum sensing from *P. aeruginosa* with garlic-derived peptides: (A) 3D interaction of VS-9 peptide with LasR protein; (B) 3D interaction of F3-3-c peptide with LasR Protein; (C) 3D interaction of TP-1 peptide with LasR protein. The top panel presents a box plot summarizing the HDOCK top 10 scores for the predicted LasR with VS-9, F3-3-c, and TP-1 complex, respectively. Middle panels show biofilm-associated surface protein (Baps) from *S. aureus* interact with garlic-derived peptides: (D) 3D interaction of VS-9 peptide with Baps protein; (E) 3D Interaction of F3-3-c peptide with Baps protein; (F) 3D interaction of TP-1 peptide with Baps protein. The bottom panel presents a box plot summarizing the HDOCK top 10 scores for the predicted Baps with VS-9, F3-3-c, and TP-1 complex, respectively. Lower panels show *SrtA* from *S. aureus* interact with garlic-derived peptides: (G) 3D interaction of VS-9 peptide with SrtA protein; (H) 3D Interaction of F3-3-c peptide with SrtA protein; (I) 3D interaction of TP-1 peptide with SrtA protein. The bottom panel presents a box plot summarizing the HDOCK top 10 scores for the predicted SrtA with VS-9, F3-3-c, and TP-1 complex, respectively.

Peptide treatments are taken to treat a variety of illnesses because they are highly selective, safe, and easily synthesized in the body.[25] In this study, the modeled garlic derivative peptides were shown to be not toxic, moderately hydrophilic and exhibiting some amphipathicity. The positive charge from Lysine, due to its positively charged chain and high isoelectric point (pI), indicates a basic nature at neutral pH. Interestingly, the slight steric barrier and side bulk values suggest possible interactions with other molecules.

Sahoo *et al.*[2] reviewed that bioinformatics methods may predict inferior physiochemical parameters for peptide drug development and therefore, saving time and resources at an early stage of development. When evaluating a peptide as a potential therapeutic candidate, its Grand Average Hydrophobicity (GRAVY), charge (isoelectric point), molecular weight, and half-life are all crucial considerations. The VS-9 peptide has good water solubility and a modest positive charge at physiological pH, which may be advantageous depending on the peptide's intended usage, such as anti-QS, according to PepCalc data. However, the lack of a strong extinction coefficient limits the use of a common technique for peptide quantification.

Peptide interactions and structures can both be analyzed computationally using techniques including homology modeling, PPI targeting, and protein docking. All the modified models show slight differences from the initial model, however, the RMSD values were all below 1 Å, indicating that the structural deviations are not substantial. Higher MolProbity scores and clash scores in some models appear to indicate suggesting geometric issues may have been introduced during the refining process. All models, however, feature well-placed side chains and Ramachandran plots. The Ramachandran plot's core and allowed regions have a substantial number of residues, which suggests that most amino acids have good backbone conformation. Furthermore, the ProSA-web results showed that, among the three peptides, VS-9 has the most favorable structure, followed by F3-3-c and TP-1, indicating a generally good structure. It is most likely more folded and more closely related to globular proteins than the others.

Docking is used to predict the interactions between molecules, to emphasize how well they fit together on different surfaces. In drug development, it is used to find compounds that can bind to particular protein locations and potentially disrupt protein-protein interactions.[45] Even though the HDOCK scores show a positive interaction between VS-9 and the target proteins, more research is required to fully understand the possible mechanisms of action of garlic peptides.

Considering that biofilm formations and antibiotic resistance are two detrimental activities that are encouraged by QS and call for novel approaches to combating microorganisms, peptides derived from garlic have been shown to inhibit foodborne bacteria, although, more studies are required to determine their exact mechanism of action.

Conclusions

Quorum sensing promotes undesirable activities including biofilm development. Garlic-derived peptides offer potential as treatments for bacterial infections. The peptides VS-9 and F3-3-c

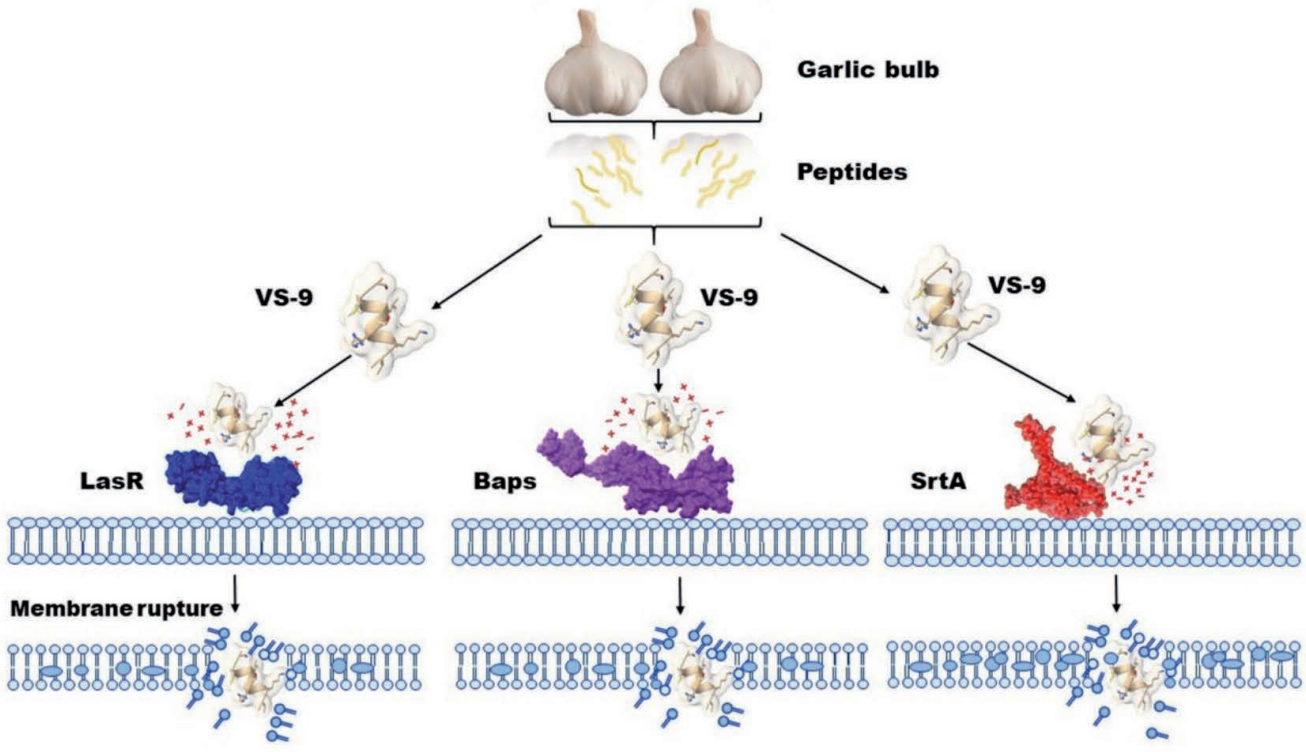

Figure 4. Proposed mechanism of garlic peptide-mediated membrane disruption and potential interaction with cell membrane-targeted proteins in Gram-positive and Gram-negative bacteria: VS-9: fresh garlic-derived peptides; LasR: proteins quorum sensing from *P. aeruginosa*; Baps: Biofilm-associated surface protein from *S. aureus*; SrtA: sortase protein from *S. aureus*.

show promise in combating foodborne illnesses, with VS-9 demonstrating elevated antibacterial activity compared to F3-3-c, using computational modeling approach. However, more research is required to fully understand how processing methods affect the biopeptide content of fresh and Laba garlic. Overcoming hurdles relating to peptide stability, transport, and the complexities of bacterial interactions is critical for successfully translating these results into therapeutic applications. Expanding our understanding of garlic-derived peptides and their interactions with bacterial targets allows us to design novel medicines to address the important issue of antibiotic-resistance and enhance human health.

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Online supplementary material:

Table S1. Source of Peptides.

Table S2. Quality of a predicted protein structure.

Table S3: Protein structure refinement using GalaxyRefine.