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Functional characterization, antimicrobial effects, and potential antibacterial mechanisms of new mastoparan peptides from hornet venom (Vespa ducalis, Vespa mandarinia, and Vespa affinis)

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\textbf{ABSTRACT}

Antibiotic-resistant bacteria are a major threat to global public health, and there is an urgent need to find effective, antimicrobial treatments that can be well tolerated by humans. Hornet venom is known to have antimicrobial properties, and contains peptides with similarity to known antimicrobial peptides (AMPs), mastoparan. We identified multiple new AMPs from the venom glands of Vespa ducalis (U-VVTX-Vm1a, U-VVTX-Vm1b, and U-VVTX-Vm1c), Vespa mandarinia (U-VVTX-Vm1d), and Vespa affinis (U-VVTX-Vm1e). All of these AMPs have highly similar sequences and are related to the toxic peptide, mastoparan. Our newly identified AMPs have \(\alpha\)-helical structures, are amphiphilic, and have antimicrobial properties. Both U-VVTX-Vm1b and U-VVTX-Vm1e killed bacteria, Staphylococcus aureus ATCC25923 and Escherichia coli ATCC25922, at the concentrations of 16 \(\mu\)g/mL and 32 \(\mu\)g/mL, respectively. None of the five AMPs exhibited strong toxicity as measured via their hemolytic activity on red blood cells. U-VVTX-Vm1b was able to increase the permeability of \(E.\) coli ATCC25922 and degrade its genomic DNA. These results are promising, demonstrate the value of investigating hornet venom as an antimicrobial treatment, and add to the growing arsenal of such naturally derived treatments.

1. Introduction

The use of antibiotics has predictably led to an increase in drug-resistant pathogens and is a serious threat to global public health. (Neu, 1992; Martínez et al., 1994; Gillings, 2013; Yang et al., 2013). The development of new antimicrobial agents is therefore necessary. Antimicrobial peptides (AMPs) are small molecule peptides that exhibit broad-spectrum and high-efficiency antibacterial activity. Just as importantly, they show low to no toxicity against red blood cells. AMPs therefore show promise as a new source of antimicrobial drugs to augment traditional antibiotics (Chen et al., 2021; Heymich et al., 2021; Zarghami et al., 2021).

In recent years, attention has focused on hornet venoms as sources of new pharmacological agents (Haberman, 1972; Pak, 2016). In particular, hornet and wasp venoms can contain the AMP mastoparan, a single-chain cationic tetradecapeptide composed of 14 amino acids, and its variants. AMPs in the mastoparan family have a wide range of applications and have shown promise in antibacterial and cancer therapies (Yamada et al., 2005; Moreno and Giralt, 2015; Silva et al., 2020).

Recently, we reported on mastoparan-like AMPs (U-VVTX-Vp1a, U-VVTX-Vp1b, U-VVTX-Vp2a, and U-VVTX-Vp2b) from the venom of \textit{V. velutina} (Meng et al., 2021). All four of these peptides showed scavenging ability against DPPH, ABTS\ &, and \(\bullet\)OH free radicals. In particular, U-VVTX-Vp1b strongly inhibited the growth of \textit{Staphylococcus aureus} and \textit{Escherichia coli} bacteria at concentrations of 60 and 120 \(\mu\)M and had low hemolytic activity.

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Here, we focused on three other social hornet species, Vespa ducalis, Vespa mandarinia and Vespa affinis, whose venoms are also rich in bioactive substances, including amines, small peptides, and high molecular weight proteins that include enzymes, allergens, and toxins. We therefore searched for novel AMPs in the venoms of V. ducalis, V. mandarinia and V. affinis and tested their antimicrobial effects, examined their toxicity to mammalian cells, and explored the toxicity mechanism of the most promising AMP.

2. Methods

2.1. Hornets and venom extraction

Vespa ducalis, V. mandarinia and V. affinis hornets (10 individuals of each species) were collected in Yunnan, China by being captured from their nests and while they were foraging. Individuals were immediately placed in liquid nitrogen. In the laboratory, they were carefully thawed on ice, and the wasp venom sacs were quickly dissected out to preserve their biological activity.

2.2. cDNA cloning and AMP identification

DNA libraries of these hornet venoms were built. We used the HIScript® II 1st Stand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd) to reverse transcribe RNA into first-strand cDNA. Double-strand cDNA was then synthesized by utilizing first-strand cDNA, a 5′-AAGCAGTGGTATCAACGCAGAGT-3′-ATGAGTGCCGAAGCTTTAGCT-3′ PCR Primer: 5′-ATTCTTAGGCAGCCGGCGCCGACATG-d(T)30N-1N-3′ (N = A, G, C, or T; N-1 = A, G, or C) in the second-strand to allow long distance PCR. Finally, this cDNA was used as a template with a 5′ primer (5′-ATGAGTGGTATCAACGCAGAGT-3′) and the 3′ SMART™ CDS III/3′ PCR Primer for PCR. We used the following settings: 1 min at 94 °C, 30 cycles for 10 s at 92 °C, 30 s at 53 °C, and 40 s at 72 °C, followed by a final 10 min at 72 °C. We used a general rTaq enzyme (TaKaRa Biotechnology Co., Ltd., Dalian, China).

The resulting PCR amplification products were then purified with a Fast Gel DNA Extraction Mini Kit (Vazyme Biotech Co., Ltd). The purified cDNA was then linked to a pMD™19-T vector (TaKaRa Biotechnology Co., Ltd.), and the ligation products were inserted into incubated DH5a competent cells for cloning and sent to TsingKe Biotechnology Co. Ltd., Dalian, China.

2.3. Properties of the new AMPs

To explore the potential properties of these newly identified AMPs, we used bioinformatics to compare them with known mastoparan-like AMPs. The physical and chemical properties of the new AMPs were predicted by ProtParam (http://web.expasy.org/protparam/) in ExPASy. The three-dimensional structures were estimated using Swiss-Model (http://swissmodel.expasy.org/) software. Helical wheel projections were made with HeliQuest (http://heliquest.ipmc.cnrs.fr/). We used ProtScale to predict the hydrophilic and hydrophobic properties of the AMPs (https://web.expasy.org/protscale/).

2.4. AMP synthesis

Using the sequences obtained, we next synthesized the putative AMPs to test their bioactivity. AMPs were synthesized by Biotech Synthetic Corporation (Bioweys Co. Ltd., China; Biochemical Co. Ltd., China) using Fmoc solid-phase peptide synthesis (Tavares et al., 2020). All the processes were carried out according to the company’s standard protocols. The molecular weight and purity of the target polypeptides were then confirmed by the company using high performance liquid chromatography-mass spectrometry (HPLC-MS).

2.5. Antimicrobial assays

We identified five new AMPs and tested their antibacterial activity with the minimum inhibitory concentration (MIC) method using broth microdilution, following the recommended methods of the American Institute of Clinical and Laboratory Standards (Ceriotti et al., 2012). Bacterial solutions were allowed to logarithmically grow in media at 37 °C until their concentrations reached the experimental requirement: OD600 = 1. We then used Mueller-Hinton liquid medium to make the inoculum solution with 106 CFU/mL. We next mixed 100 μL of this diluted bacterial solution with 100 μL of each AMP. For each AMP we tested the efficacy of nine different concentrations: 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg/mL. The blank control consisted of only 200 μL diluted bacterial solution (no AMPs added). All bacteria were then incubated at 37 °C for 16–18 h. The MICs were determined by measuring the OD600 using standard methods (Jimenez Vargas et al., 2021). Each concentration of each AMP was replicated three times in parallel. Final results were obtained from three independent trials. The bactericidal rate was calculated with equation (1).

\[
\text{Bacteriostatic rate} = \left(1 - \frac{A_{\text{peptide}}}{A_{\text{blank}}}\right) \times 100 \quad \text{Equation 1}
\]

2.6. Hemolysis assays

Hemolysis of red blood cells can be used to measure the toxicity of an antimicrobial treatment. We used a slight modification of the classical method (Watala and Kowalczyk, 1990; Baek et al., 2011). We centrifuged 1 mL of sheep blood to obtain an erythrocyte precipitate. The resulting red blood cells were diluted to 2% in an isotonic solution. Each of the test AMPs was then added (concentration of 256 μg/mL) to the diluted red blood cell suspension and incubated at 37 °C for 30 min. The supernatants were then placed into the wells of a 96-well plate to measure their optical density at 540 nm (OD540). The positive control was 0.1% Triton X-100 and the blank control was 1 × PBS. Final results were obtained from three independent trials. Equation (2) shows how we calculated the hemolysis rate.

\[
\text{Hemolysis rate} = \left(1 - \frac{A_{\text{peptide}} - A_{\text{PBS}}}{A_{0.1\% \text{ Triton X-100}} - A_{\text{PBS}}}\right) \times 100 \quad \text{Equation 2}
\]

2.7. Mechanism of bacterial damage

2.7.1. Bacterial membrane permeability assay

We next tested if the MIC of AMP U-VVTX-Vm1b could rupture bacterial cell membranes by using propidium iodide (PI) to stain the DNA inside ruptured cells. Escherichia coli ATCC25922 was treated with the MIC of AMP U-VVTX-Vm1b for 2 h at 37 °C. PI (100 μg/mL) was added to the mixture, which was further incubated for 10 min at 37 °C. Fluorescence was then observed with a laser scanning confocal microscope (Olympus Fluoview™ FV1000, Japan) to measure the degree of bacterial membrane permeability (Zhang et al., 2015).

2.7.2. Electrophoretic mobility shift assays (EMSA)

To test if AMP U-VVTX-Vm1b could degrade bacterial DNA, we used an Electrophoretic Mobility Shift Assay (EMSA) (Bandyopadhyay et al., 2013). DNA from E. coli ATCC25922 was extracted with a bacterial genomic DNA extraction kit (Vazyme Biotech Co., Ltd). We placed the AMP in a buffer (testing nine AMP concentrations: 1, 2, 4, 8, 16, 32, 64, 128 and 256 μg/mL) and tested each concentration with an equal volume of bacterial DNA in buffer. The blank control was added with an equal volume of 1 × PBS (marked 0 μg/mL). We incubated the solutions at 37 °C for 3 h. We used an agarose gel containing 1 μL Gel Stain with a concentration of 2% and ran the different solutions for 35 min at 120 V.
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Fig. 1. Sequences of mastoparan-like AMPs from V. ducalis, V. mandarinia and V. affinis. (A) Nucleotide and amino acid sequences of U-VVTX-Vm1a are shown as a representative example. The mature AMP sequence is marked in italic and bold type. The stop codon of the sequence is shown with an asterisk. (B) Amino acid sequences of mastoparan-T1 to -T3 and the five new AMPs that we identified. Mature AMP sequences are marked in italic and bold type. The amino acids that differ between the newly identified AMPs are marked in red.

Table 1
Physical and chemical properties of five new mastoparan-like AMPs from V. ducalis, V. mandarinia and V. affinis.

<table>
<thead>
<tr>
<th>AMPs</th>
<th>Theoretical molecular weight (Da)</th>
<th>Number of amino acids</th>
<th>Net charge</th>
<th>Grand average of hydropathicity</th>
<th>Instability index</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-VVTX-Vm1a</td>
<td>1453.83</td>
<td>14</td>
<td>3</td>
<td>0.829</td>
<td>-2.84</td>
</tr>
<tr>
<td>U-VVTX-Vm1b</td>
<td>1507.93</td>
<td>14</td>
<td>3</td>
<td>1.114</td>
<td>16.98</td>
</tr>
<tr>
<td>U-VVTX-Vm1c</td>
<td>1465.89</td>
<td>14</td>
<td>3</td>
<td>1.136</td>
<td>-2.84</td>
</tr>
<tr>
<td>U-VVTX-Vm1d</td>
<td>1465.89</td>
<td>14</td>
<td>3</td>
<td>1.000</td>
<td>8.91</td>
</tr>
<tr>
<td>U-VVTX-Vm1e</td>
<td>1541.94</td>
<td>14</td>
<td>3</td>
<td>1.043</td>
<td>16.98</td>
</tr>
</tbody>
</table>

Fig. 2. Structure and hydrophobicity of a typical AMP that we identified, U-VVTX-Vm1a from V. ducalis. (A) Helical wheel projection with hydrophobic residues such as Ala (A), Phe (F), Ile (I), and Leu (L) residues shown in yellow. Hydrophilic residues such as Arg (R) and Lys (K) are marked in blue. (B) Hydrophobicity analysis in which scores from positions 5-11 represent precursors of U-VVTX-Vm1a, and scores of positions 22-35 represent mature sequences of this AMP. The N-terminal signal region of this AMP is located at position 22, and position 35 is a C-terminal tail domain. Positive scores indicate hydrophobicity and negative scores suggest hydrophilia. (C) The theoretical three-dimensional structure of this AMP. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
3. Results

3.1. Identification of mastoparan-like AMPs from cDNA

Eight similar sequences were obtained using DNAMAN 9.0 (Lynnon Biosoft company, USA) software by comparing the cloned 158 cDNA sequences that we obtained (TsingKe Biotechnology Co. Ltd., China). Five new sequences were identified by using a protein-protein BLAST (BlastP) search in the NCBI database. We named the AMPs as follows: U-VVTX-Vm1a (accession: MZ323231), U-VVTX-Vm1b (accession: MZ323232) and U-VVTX-Vm1c (accession: MZ323233) came from the venom glands of *V. ducalis*; U-VVTX-Vm1d (accession: MZ323234) was from the venom of *V. mandarinia*; and U-VVTX-Vm1e (accession: MZ323235) was from the venom of *V. affinis*. These five AMP sequences are quite similar to previously reported sequences (Fig. 1A, in which U-VVTX-Vm1a is shown as a representative example). In Fig. 1B, we show the amino acid sequences of all five new AMPs and compare them to previously identified mastoparan-like AMPs.

![Fig. 3. Properties of U-VVTX-Vm1a: (A) HPLC profile and (B) MS spectra.](image)

3.2. Properties of the new AMPs

The five AMPs (U-VVTX-Vm1a to -Vm1e) that we identified have similar properties (Table 1). The theoretical molecular weight of each AMP is approximately 1500 Da, and all AMPs have three positive charges (Wang, 2015). The instability indexes of these AMPs are all less than 40, which indicates that they are stable proteins. The grand average hydropathicities were all positive, suggesting that these compounds have some hydrophobicity.

In Fig. 2, we show details of the hydrophobicity and structure of a typical AMP that we identified, U-VVTX-Vm1a from *V. ducalis*. There are both hydrophilic (N-terminal) and hydrophobic (C-terminal) regions, suggesting that this AMPs could be amphiphilic (Kazuma et al., 2017). Hydrophilic and hydrophobic regions were likewise found in all our AMPs. This result is in line with the amphiphilicity reported for all previously identified AMPs (Devine and Hancock, 2002). Modeling suggests that all of our newly identified AMPs have an \( \alpha \)-helical structure, like all mastoparan-like AMPs. The similarities between the
hydrophobicity and structures of our newly identified AMPs and mastoparan-like AMPs further suggests that our AMPs are antimicrobial peptides.

3.3. New AMPs synthesis

The results of HPLC and MS for U-VVTX-Vm1a, a typical AMP, are shown in Fig. 3. The corresponding results for the other four new AMPs are shown in the supplemental material. The purities were analyzed by HPLC, and the molecular weights of the AMPs was determined by MALDI-TOF-MS. In brief, the purity of our five new AMPs following solid-phase synthesis was >98%. The actual average molecular weights of each AMP (measured via mass spectrometry) were 1453.81, 1507.9, 1465.86, 1465.86 and 1541.92 Da for U-VVTX-Vm1a, Vm1b, Vm1c, Vm1d, and Vm1e, respectively. This is consistent with the theoretical molecular weights shown in Table 1.

3.4. Antimicrobial activities

The MICs of all five novel AMPs are shown in Table 2. None of these five AMPs killed *P. aeruginosa* CGMCC1.10712. However, all AMPs (U-VVTX-Vm1a to -Vm1e) had some ability to kill gram-positive and gram-negative bacteria. U-VVTX-Vm1b and U-VVTX-Vm1e also inhibited the growth of *C. albicans* CA10231. Notably, U-VVTX-Vm1b and U-VVTX-Vm1e had the strongest bactericidal effect, with MICs at the lower AMP concentrations of 16 μg/mL and 32 μg/mL, respectively.

3.5. Hemolytic activities

All the tested AMPs showed almost no hemolysis of red blood cells. The hemolysis rates of AMPs (U-VVTX-Vm1a to -Vm1e) were all less than 5% and ranged from 2.29 to 3.97% (Table 2).

3.6. Mechanism of bacterial damage

Because U-VVTX-Vm1b showed the strongest antibacterial effect, we explored how it may kill bacteria. PI staining demonstrated that *E. coli* ATCC25922 incubated with U-VVTX-Vm1b at its MIC had damaged cell membranes (showing red fluorescence), whereas no controls exhibited any red fluorescence (Fig. 4a, Fig. 4b). U-VVTX-Vm1b also degraded *E. coli* ATCC25922 genomic DNA (Fig. 4c). When the concentration of U-VVTX-Vm1b was at any of the tested concentrations (1–256 μg/mL), the DNA bands were more dispersed and less intense in comparison with the control genomic DNA band (marked 0 in Fig. 4c), which was clear and bright without migration or dispersion. In particular, the three highest concentrations of the AMP (64, 128 and 256 μg/mL) resulted in far less intense bacterial DNA bands (Fig. 4c). These results show that U-VVTX-Vm1b can degrade *E. coli* cell membranes and DNA, providing a potential mechanism for its antimicrobial effect.

### Table 2

Antibacterial, antifungal, and hemolytic activities of the new AMPs. In the table header, for brevity, we show the AMP-specific suffix. The prefix for all AMPs is “U-VVTX-“.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Vm1a</th>
<th>Vm1b</th>
<th>Vm1c</th>
<th>Vm1d</th>
<th>Vm1e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC25923</td>
<td>256</td>
<td>16</td>
<td>128</td>
<td>256</td>
<td>32</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> 168</td>
<td>256</td>
<td>128</td>
<td>256</td>
<td>ND</td>
<td>64</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC29212</td>
<td>256</td>
<td>256</td>
<td>256</td>
<td>ND</td>
<td>256</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC25922</td>
<td>256</td>
<td>16</td>
<td>128</td>
<td>256</td>
<td>32</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> CGMCC 1.10712</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em> CA10231</td>
<td>ND</td>
<td>64</td>
<td>ND</td>
<td>ND</td>
<td>256</td>
</tr>
<tr>
<td>Red blood cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The sheep erythrocytes</td>
<td>3.033 ± 2.994</td>
<td>3.362 ± 3.970</td>
<td>3.548 ± 3.548</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>The sheep erythrocytes</td>
<td>0.008</td>
<td>0.005</td>
<td>0.008</td>
<td>0.005</td>
<td>0.008</td>
</tr>
</tbody>
</table>

* Minimum dosage of AMPs for completely inhibiting bacterial growth.

b Hemolysis rate of red blood cells with the maximum concentration of 256 μg/mL. ND: no detectable antibiotic or antifungal action at a concentration of 256 μg/mL.

---

Fig. 4. The membrane permeability of *E. coli* ATCC25922. Propidium iodide (PI) increased with exposure to U-VVTX-Vm1b, indicating membrane damage: (a) is the blank control group (1 × PBS), (b) is the treatment group incubated with U-VVTX-Vm1b at 1 × MIC. (c): Gel blocking analysis of U-VVTX-Vm1b binding to *E. coli* ATCC25922. Successively higher concentrations of U-VVTX-Vm1b (numbers correspond to units of μg/mL) were added to the DNA of *E. coli* ATCC25922. The blank control was added with equal volume of 1 × PBS (marked 0 μg/mL).
4. Discussion

There is growing interest in searching for natural antimicrobial compounds from hornet and wasp venoms. We used cDNA cloning and mastoparan primers to obtain five new AMPs (U-VVTX-Vm1a to -Vm1e) from the venom of V. ducaIis, V. mandarinia and V. affinis. The homology between these AMPs was high, with the highest similarity at 92.86%. The new AMPs that we identified are closely related to mastoparan-like peptide 12d (accession P0CG7), mastoparan-M (accession P04205), mastoparan-like peptide 12c (accession 05PS40), mastoparan-T (accession P0C1Q7), and prepromastoparan D (accession HQ168024) (Hirai et al., 1981; Ho and Hwang, 1991; King et al., 2003; Xu et al., 2006). This similarity between our newly identified AMPs and these previously identified mastoparan-like peptides is not surprising because we used primers for mastoparan-like peptides, but does demonstrate the efficacy of this screening method.

We then used bioinformatics to predict the structure and function of the five new AMPs. Likewise, the physical and chemical properties and amphiphilic characteristics of our AMPs are similar to those of mastoparan-like peptides and are predicted to share a typical mastoparan-like α-helix structure. Antimicrobial peptides with an α-helix structure are widely distributed in nature and have relatively high antimicrobial activity (Argiolas and Pisano, 1984; Souza et al., 2005; Rangel et al., 2011; Lee et al., 2016; Choi and Lee, 2020). Our results support the hypothesis that U-VVTX-Vm1a to -Vm1e are antimicrobial peptide analogs of mastoparan and should be bactericidal (Lin et al., 2011).

We synthesized our five new AMPs and tested their antimicrobial activity. In our study, none of our AMPs killed P. aeruginosa CGMCC 1.10712 perhaps because amidation of mastoparan-like peptides at their C-terminals is related to their broad-spectrum antimicrobial effects (Hara and Yamakawa, 1995). Based on the translated cDNA sequences (Fig. 1) and what is known about homologous peptides, such peptides with amidated C-termini are predicted to exist in hornet venoms. However, our synthetic antimicrobial peptides were not modified with amides at their C-terminals.

Nonetheless, U-VVTX-Vm1b (final MIC of 16 μg/mL) and U-VVTX-Vm1e (final MIC of 32 μg/mL) exhibited fairly strong antibacterial activity against various standard and drug-resistant bacterial strains. The other AMPs that we identified (U-VVTX-Vm1a, U-VVTX-Vm1c and U-VVTX-Vm1d) showed some antibacterial activity at higher MICs.

The higher efficacy of U-VVTX-Vm1b and U-VVTX-Vm1e may be related to both of these peptides having an arginine on the fourth residue of their mature sequences, something not found in the other three AMPs that we identified (Fig. 1). The effects of the different amino acid components of wasp venom AMPs upon their antimicrobial abilities is unclear. It would be interesting to conduct a broader study of the sequences of all the different venom AMPs that have antimicrobial effects. New data on mastoparan-like AMPs (U-VVTX-Vp1a, U-VVTX-Vp1b, U-VVTX-Vp2a, and U-VVTX-Vp2b) from the venom of V. velutina could help inform this effort (Meng et al., 2021). Peptides with similar sequences have been found in the venom of wasps such as Eumenes pomiformis and Orancistrocerus dresensi (Baek and Lee, 2010; Lee et al., 2016; Konno et al., 2019). The AMPs we identified are also similar to peptides reported previously (mastoparan II from the venom of V. orientalis, mastoparan C isolated from the venom of V. crabo, mastoparan V isolated from the venom of V. velutina, and mastoparan X isolated from the venom of V. xanthoptera), although there are key differences in amino acids at different locations (Hirai et al., 1979; Lee et al., 2016; Lin et al., 2017; Chen et al., 2018). This diversity of mastoparans suggests that researchers could use sequence comparison and conformational analyses, and replace key amino acids to test the importance of different amino acids at specific locations and to potentially increase the antimicrobial activity of these peptides.

We focused on potential mechanisms for the antibacterial activity of U-VVTX-Vm1b because it had the lowest MIC (16 μg/mL). We found that this AMP could increase the permeability of E. coli cell membranes and degrade E. coli genomic DNA. The positive charge carried by U-VVTX-Vm1b and the negatively charged lipopolysaccharide or teichoic acid on the bacterial cell membrane likely allows the AMP to penetrate the cell’s peptidoglycan layer and electrostatically attach to the plasma membrane. The AMP could then form transmembrane channels, leading to leakage of nucleic acids and cell lysis from osmotic forces (Toke, 2005; Ageitos et al., 2017). This general mechanism may apply to multiple AMPs. Yaghoubi et al. (2021) found that a novel antimicrobial peptide likewise increases the permeability of bacteria and cancer cell membranes, thereby killing these cells. Additional studies of these mechanisms would be illuminating.

In conclusion, the search for AMPs in wasp and hornet venom has practical benefits for developing new antibacterial treatments, particularly against antibiotic-resistant bacterial strains, and potential for new anti-cancer treatments. Given the sequence and structural homologies among these antibacterial peptides and their variation across multiple species, such studies may also shed light on the importance of different amino acid substitutions and suggest strategies for the intelligent design of even more effective AMPs.