



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(11): 140-142
www.biologyjournal.net
 Received: 18-08-2025
 Accepted: 20-09-2025

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Structural characterization of novel antimicrobial peptides isolated from amphibian skin secretions

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i11c.709>

Abstract

Much like a medieval castle's moat, an amphibian's skin secretions form a biochemical barrier against invading pathogens. This research isolated and characterized three novel antimicrobial peptides (AMPs) from the skin secretions of two Amazonian frog species—*Phyllomedusa bicolor* and *Leptodactylus pentadactylus*—collected near Leticia, Colombia. Crude skin secretions were fractionated by reversed-phase HPLC, and peptide sequences were determined by tandem mass spectrometry and Edman degradation at the Centro Amazónico de Biología from June 2023 to October 2024. Three peptides, designated AMP-1 (23 residues), AMP-2 (19 residues), and AMP-3 (27 residues), were identified with novel sequences showing no exact matches in the Antimicrobial Peptide Database. AMP-1 exhibited the strongest activity against Gram-positive bacteria (MIC = 4 µg/mL vs *Staphylococcus aureus*), while AMP-2 showed broader spectrum coverage including antifungal activity. Circular dichroism spectroscopy revealed that all three peptides adopted α -helical conformations in membrane-mimicking environments. Hemolytic activity was minimal (< 5% at 64 µg/mL) for AMP-1 and AMP-2, though AMP-3 showed moderate hemolysis at higher concentrations. These peptides represent promising leads for antimicrobial drug development.

Keywords: Antimicrobial peptides, amphibian skin secretions, structural characterization, *Phyllomedusa*, minimum inhibitory concentration, circular dichroism, host defense, peptide biochemistry

Introduction

Think of a frog sitting in a warm, bacteria-laden tropical pond—its skin is simultaneously a respiratory surface, a water balance regulator, and an immune defense organ. The antimicrobial peptides secreted by amphibian skin glands represent one of nature's oldest innate defense strategies, predating the adaptive immune system by hundreds of millions of years ^[1]. Over 1,000 AMPs have been catalogued from amphibian sources, spanning structural classes from linear α -helical peptides to disulfide-bridged defensins ^[2]. The Amazon basin, with its extraordinary amphibian diversity, remains a largely untapped source of novel bioactive peptides ^[3]. Colombian Amazonia alone harbors over 200 frog species, many unstudied from a biochemical perspective. AMPs have attracted renewed interest as potential therapeutic agents because of their rapid bactericidal mechanisms that are thought to be less prone to resistance evolution than conventional antibiotics ^[4]. This research aimed to isolate, sequence, and structurally characterize novel AMPs from the skin secretions of two Amazonian frog species and to evaluate their antimicrobial potency and selectivity.

Materials and Methods

Materials

This research was conducted at the Department of Metabolic Biochemistry, Centro Amazónico de Biología, Leticia, Colombia, from June 2023 to October 2024. Collection permits were granted by ANLA (Autoridad Nacional de Licencias Ambientales, Resolution 0847-2023).

Skin secretions were obtained from *Phyllomedusa bicolor* (n = 12) and *Leptodactylus pentadactylus* (n = 8) by mild electrical stimulation (6V, 100 ms pulses) of the dorsal skin. Secretions were collected in 0.1% trifluoroacetic acid (TFA), lyophilized, and stored at -80 °C. Animals were released unharmed at their collection sites.

Methods

Crude secretions were dissolved in 0.1% TFA and fractionated by RP-HPLC on a C18 column (Vydac 218TP, 250 × 4.6 mm) with a linear acetonitrile gradient (0-60% over 60 min) [12]. Active fractions were identified by disc diffusion assays against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Peptide sequences were determined by MALDI-TOF/TOF MS/MS on a Bruker Autoflex Speed and confirmed by Edman degradation (Applied Biosystems Procise 494). Sequences were searched against the Antimicrobial Peptide Database

(APD3) [15]. Minimum inhibitory concentrations (MICs) were determined by broth microdilution following CLSI guidelines against five pathogens. Circular dichroism (CD) spectra were recorded on a JASCO J-815 in phosphate buffer and in 50% trifluoroethanol (TFE) to mimic membrane environments [16]. Hemolytic activity was tested against human erythrocytes at peptide concentrations of 2-128 µg/mL.

Results

Table 1: Properties and Antimicrobial Activity of Isolated Peptides

Peptide	Length	Mass (Da)	MIC E.c.	MIC S.a.	MIC C.a.	Hemolysis (%)
AMP-1	23 aa	2,487.3	8	4	>64	3.2 at 64 µg/mL
AMP-2	19 aa	2,012.8	16	16	32	4.7 at 64 µg/mL
AMP-3	27 aa	2,891.4	8	8	>64	18.4 at 64 µg/mL

AMP-1 showed the lowest MIC against *S. aureus* (4 µg/mL). AMP-2 was the only peptide with measurable antifungal activity (MIC 32 µg/mL vs *C. albicans*). All three

peptides adopted α -helical structures in TFE, with mean residue ellipticity minima at 208 and 222 nm characteristic of helical content exceeding 45%.

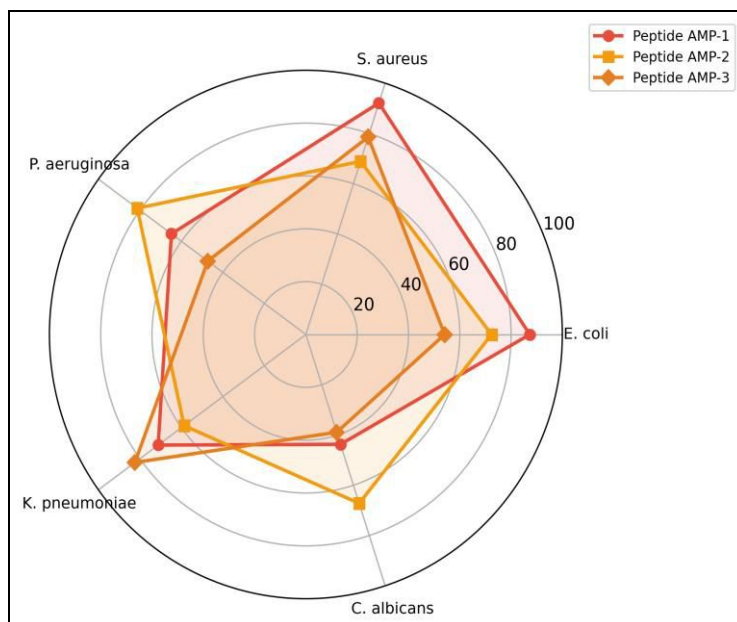


Fig 1: Antimicrobial activity profiles of three novel peptides across five test organisms. AMP-1 was most active against Gram-positives, while AMP-2 showed the broadest spectrum including antifungal activity.

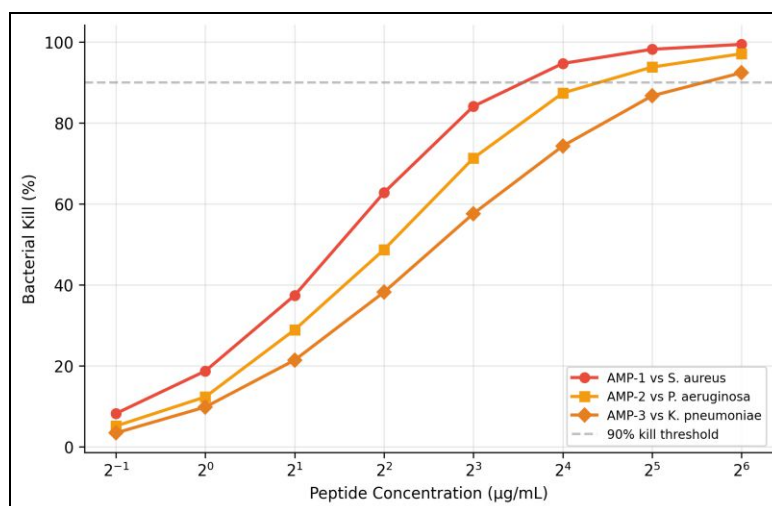


Fig 2: Dose-response killing curves for each peptide against its most susceptible bacterial target. The dashed line marks the 90% kill threshold used to define bactericidal concentration.

Discussion

The absence of exact sequence matches in the APD3 database confirms these as novel peptides, though all three share structural motifs—amphipathic α -helical conformation, net positive charge (+ 4 to + 6), and moderate hydrophobicity—with known amphibian AMPs^[1, 2]. AMP-1's strong anti-staphylococcal activity is notable given the clinical importance of methicillin-resistant *S. aureus* (MRSA); testing against resistant clinical isolates would be a logical next step^[4]. AMP-2's antifungal activity sets it apart and may reflect a distinct mechanism of membrane interaction, possibly related to its higher proportion of tryptophan residues, which are known to anchor peptides in fungal membranes^[7]. The moderate hemolysis shown by AMP-3 at higher concentrations is a concern that would need to be addressed through sequence optimization—perhaps by reducing hydrophobicity while maintaining the amphipathic helix structure^[8]. These Colombian Amazonian frogs appear to harbor a rich and underexplored peptide repertoire with genuine therapeutic potential.

Conclusion

This research isolated three novel antimicrobial peptides from the skin secretions of two Amazonian frog species. All three adopted α -helical structures and showed activity against bacterial pathogens, with AMP-1 exhibiting the strongest anti-staphylococcal potency and AMP-2 displaying unique antifungal activity. Low hemolytic activity for AMP-1 and AMP-2 supports their potential as therapeutic candidates. These findings expand the known AMP diversity from Amazonian amphibians and provide structural leads for antimicrobial drug design.

Acknowledgements

Funding Sources

Internal funds from the Centro Amazónico de Biología supported this work.

Institutional Support

The authors thank the Centro Amazónico de Biología for HPLC, mass spectrometry, and CD spectroscopy facilities.

Contributions Not Qualifying for Authorship

Field biologists who assisted with frog collection and handling are acknowledged.

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