Engineering of a novel skin secretion peptide of an endemic amphibian of Ecuador (*Callimedusa ecuatoriana*) into promising antimicrobial molecules.

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ABSTRACT

Amphibian skin secretion has been an important source of broad-spectrum and membrane-targeting antimicrobial peptides, which promise to tackle the antibiotic resistance crisis. *Callimedusa ecuatoriana* from Ecuador is an example of an unexplored species, that can hold a library of novel chemical scaffolds with antibiotic action. In this study, we report a novel skin peptide (PTR-CE1) identified by molecular cloning of mRNA precursor. We demonstrated that it lacks of antimicrobial activity. So, using the natural sequence of PTR-CE1 as a template, we designed and synthesized two analogs (PTR-CE1a and PTR-CE1b). Both engineered peptides displayed high antibacterial activity, even against the ampicillin-resistant bacterial strains. While PTR-CE1b showed MIC values of 106.5-212.99 mM and less than 10% of damage to red blood cells at 3.02 mM, PTR-CE1a displayed a more potent broad-spectrum effect against all the tested microorganisms, with MIC values of 3.02-12.06 mM, and low hemolytic properties at 6.66 mM. This study highlights the role of the secondary structure for antimicrobial activity and shows how inactive peptides can be useful as a template for the generation of new molecules with high activity and low toxicity.

BACKGROUND

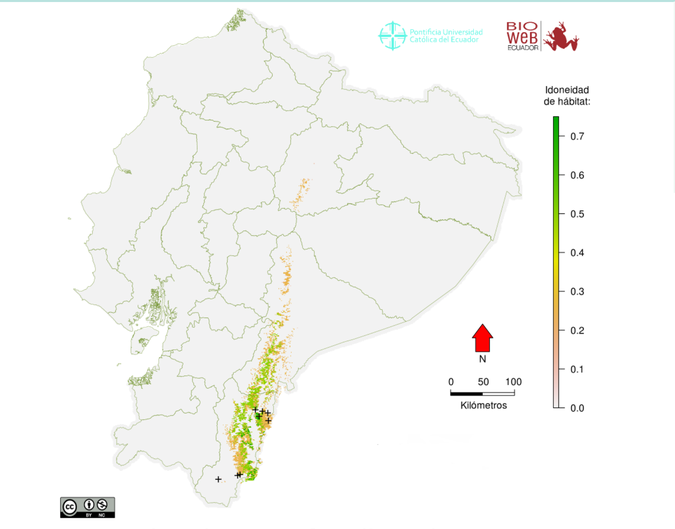
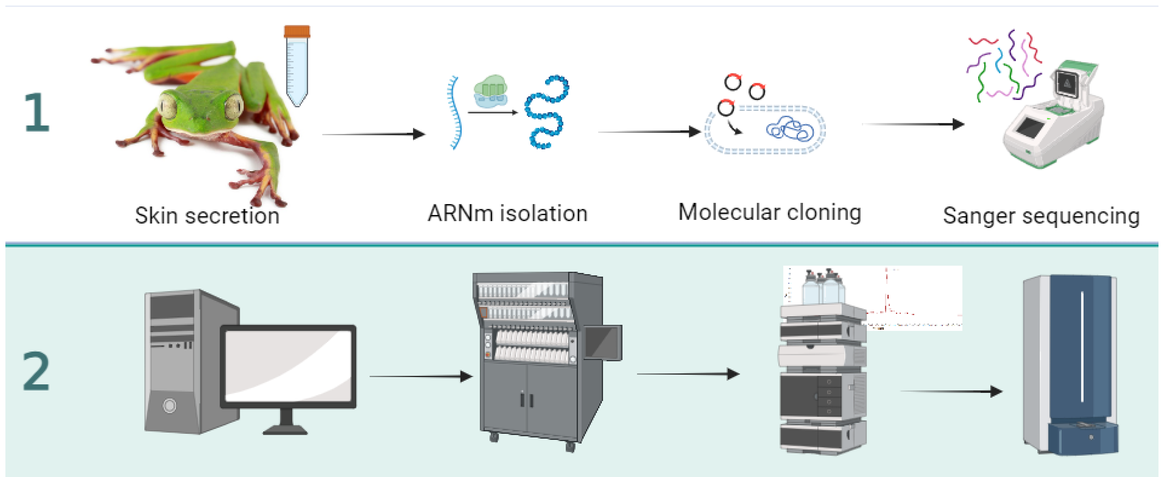


Fig 1. Map distribution of *Callimedusa ecuatoriana* in Ecuador.

- Skin frog secretions: Big source of interesting molecules.
- AMPs: ~3569 from amphibians.
- Hylidae: >200 AMPs reported.

Fig 2. *Callimedusa ecuatoriana* individual.

METHODS



RESULTS

Table 1. Physicochemical properties and 3D structure of PTR-CE1 and its analogues.

Peptide	Sequence	#Aas	Alpha helix (%)	H	mH	Net charge Z	Theoretical mass (Da)	3D Structure
PTR-CE1	GVFKDALKQFGAALPDKAANALKPK a	25	80	0.236	0.506	3	2599.07	
PTR-CE1a	GVFKKALKQFGAALRLAANALKPK a	25	88	0.364	0.462	7	2653.30	
PTR-CE1b	GVFKDALKQFGAAL-DKAANALK-K a	23	100	0.193	0.468	4	2403.85	

a= Amidated C-terminal region. Gray: Conserved sites. Black: Deletion and substitutes in analogs. H: Hidrophobicity. mH: Hydrophobic moment.

Antimicrobial activity

Table 2. Minimal inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC) of PTR-CE1 and analogs.

Synthetic peptide	MIC (µM) / MBC (µM)					
	E. coli 25922	*S. aureus 25923*	*C. albicans*	*K.pneumoniae*	*P.aeruginosa*	*B.subtilis*
Picturin-CE1	>196,99	>196,99	16	>196,99	>196,99	>196,99
	>196,99	>196,99	>196,99	>196,99	>196,99	>196,99
Picturin-CE1a	3,02	6,03	12,06	6,03	12,06	3,02
	6,03	12,06	192,96	12,06	48.24	24,12
Picturin-CE1b	53,25	>212,99	212,99	53,25	26,62	26,62
	106,49	>212,99	>212,99	106,49	>212,99	53,25
Ampicillin	46	<11	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND
Fluconazole	ND	ND	209	ND	ND	ND
	ND	ND	ND	ND	ND	ND

ND= No data.
*Ampicillin-resistant

Molecular docking-Interaction with bacterial cell membrane

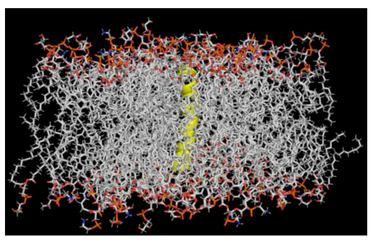


Fig 3. Docking interactions of PTR-CE1a Score of -8.4 kcal/mol

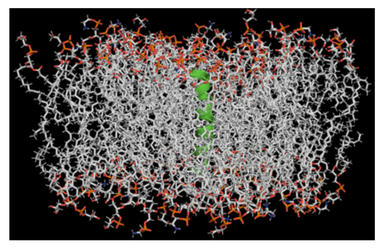


Fig 4. Docking interactions of PTR-CE1b. Score of -4.7 kcal/mol.

Hemolytic activity

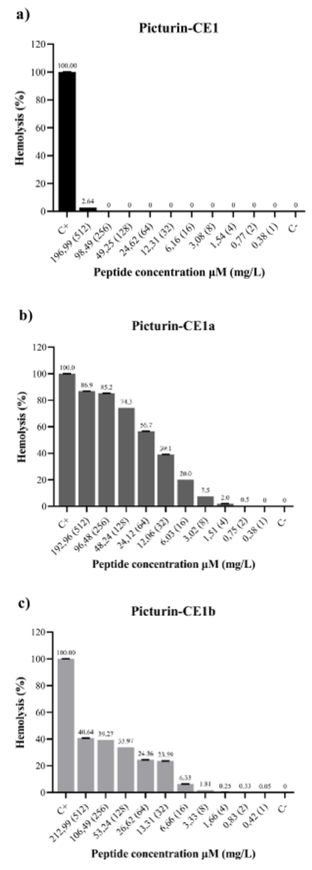


Fig 5. Hemolytic activity of PTR-CE1 and analogs. C+: Triton X-100. C-: PBS1X.

CONCLUSIONS

Peptide design based on templates with non-antibacterial activity can succesfully be transformed into bioactive agents. PTR-CE1a is a promising peptide that could be considered to fight even against antibiotic-resistant bacteria.

REFERENCES

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