Biotechnology of Macromolecules

Reptile Cathelicidins And Their Potential As New Antimicrobial Compounds

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INTRODUCTION

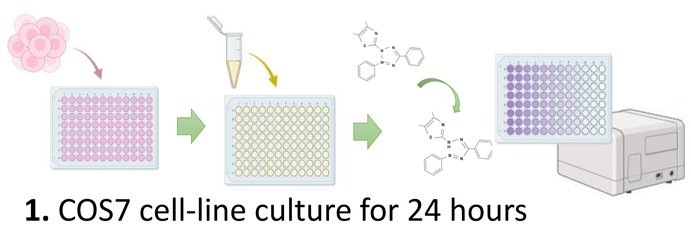
Cathelicidins are a family of host defense peptides (HDPs) with diverse functions, characterized mainly by their antimicrobial activity and found in most vertebrates [1]. Reptile cathelicidins exhibit substantial potential as a valuable source of peptides for potential biomedical applications. These peptides exhibit considerable variability and differ in sequence, structure, and function in different animal species [2]. Nevertheless, the peptides derived from these reptilian species have demonstrated remarkable antimicrobial properties. The cathelicidins found in reptiles have been subjected to limited investigation. The aim of this study was to investigate the antimicrobial properties of 4 reptile cathelicidins.

METHODS

Bioinformatic analysis

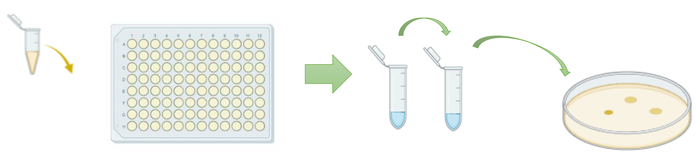
- 3D modeling of the peptide structure
- Prediction of hydrophobicity and charge (PepCalc.com)

MTT cytotoxicity assay



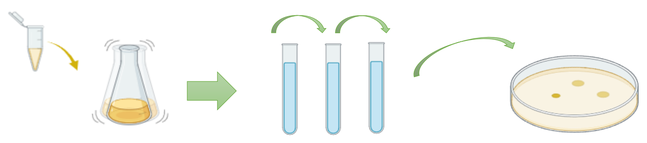
1. COS7 cell-line culture for 24 hours
2. Cells were treated with the peptides ranging from 25 µM to 0.4 µM for 72 hours
3. Cells were treated with MTT, and the absorbance was measured at 492 nm

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)



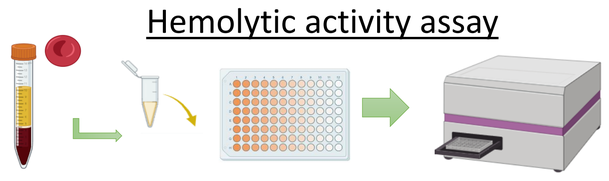
1. Peptides from 50 µM to 0.2µM were incubated with the microorganism at 1 x 10^5 CFU/ml for 18 hours
2. Dilutions and culture of wells without turbidity
3. MIC: Same bacterial concentration as in the initial inoculum. MBC: 0,1% of the initial bacterial inoculum

Time-killing curves



Bacteria at 1 x 10^5 CFU/ml was cultured using MIC of the peptide, with samples being inoculated at hourly intervals over a period of 9 hours. The assay were also conducted using the microorganism culture in exponential growth phase

Hemolytic activity assay



1. Incubation of rat erythrocytes with peptides in a range from 50 to 0.1 µM for 1 hour
2. Centrifugation of the multi-well plate and measurement of the supernatant absorbance at 405 nm

RESULTS

Seven new reptile cathelicidins were synthesized to test their *in vitro* activity. Vipericidin OH was used as the reference peptide for reptiles. All Peptides showed a net cationic charge and approximately 35% hydrophobicity (*Table 1*). Its structure is alpha helix, or two alpha helices joined by a central loop (*Figure 1*).

Peptide	OH	La	Ts	GG-RR	CC	GG-GL	Ge	Am
Charge (pH7)	15	5	11	14,1	13,1	5	6,1	5,1
Hydrophobicity	44.12%	40,91%	36,36%	44,74%	33,33%	36,36%	40,74%	46,15%

Table 1. Physicochemical properties of peptides.

All peptides, except GG-GL and Ts, showed high antibacterial activity with a MIC and MBC ranging from 0.39 to 6 µM for most microorganisms (*Table 2*). The peptides did not show antifungal activity against the yeast *C. albicans.*

			Microorganism					
			E. coli	*S. enterica*	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*C. albicans*
Peptide	OH	MIC (µM)	0,19-0,39	1,56-6,12	0,78	12,5-25	0,19-0,39	>50
		MBC (µM)	0,19-0,39	1,56-6,12	3,12-6,25	12,5-25	0,19-0,39	>50
	La	MIC (µM)	1,56-3,12	12,5	1,56-3,12	6,25-12,5	12,5-25	>50
		MBC (µM)	3,12	25-50	1,56-3,12	6,25-12,5	12,5-25	>50
	Ts	MIC (µM)	>50	>50	>50	>50	>50	>50
		MBC (µM)	>50	>50	>50	>50	>50	>50
	GG-RR	MIC (µM)	0,78-1,56	0,19-0,39	1,56-3,12	>50	0,78-1,56	>50
		MBC (µM)	1,56	0,19-0,39	3,12-6,25	>50	0,78-1,56	>50
	CC	MIC (µM)	0,78-1,56	0,78-1,56	0,78-1,56	25-50	0,78-1,56	>50
		MBC (µM)	0,78-1,56	0,78-1,56	0,78-1,56	25-50	1,56	>50
	GG-GL	MIC (µM)	25-50	>50	12,5	25-50	>50	>50
		MBC (µM)	25-50	>50	12,5-25	25-50	>50	>50
	Ge	MIC (µM)	3,12-6,25	3,12-6,25	0,78-1,56	3,12-6,25	3,12-6,25	25-50
		MBC (µM)	3,12-6,25	3,12-6,25	0,78-1,56	3,12-6,25	3,12-6,25	25-50
	Am	MIC (µM)	0,78-1,56	0,78-1,56	0,78-1,56	3,12-6,25	3,12-6,25	>50
		MBC (µM)	0,78-1,56	1,56	1,56-3,12	6,25-12,5	3,12-6,25	>50

Table 2. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of peptides.

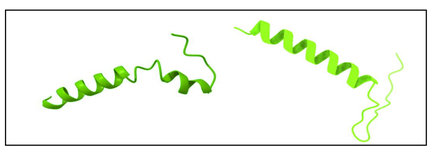


Figure 1. Three-dimensional structure prediction of peptides.

Time-killing curves were performed with the most active peptides (Ge and GG-RR). Both peptides were bactericidal when the microorganism was in the latency phase and bacteriostatic when it was in the exponential phase (*Figure 2*). Therefore, both peptides showed sensitivity to the inoculum size and had an optimal activity range of 6 hours.

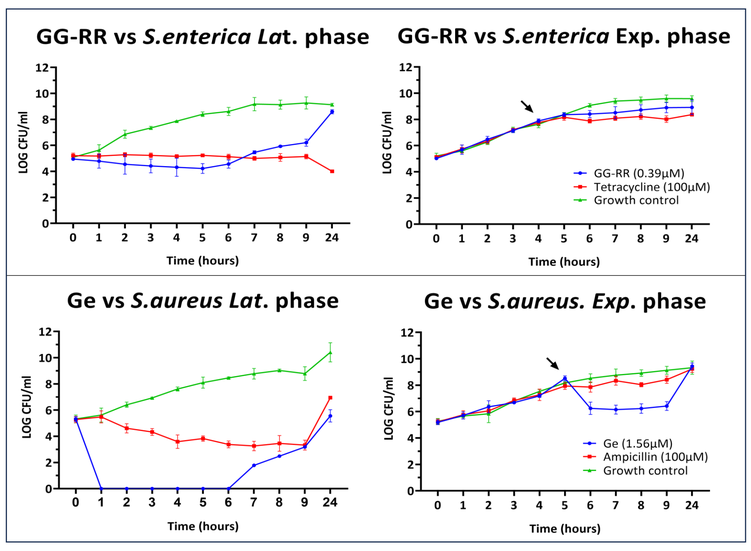


Figure 2. Time-killing curves of Ge and GG-RR peptides against *S. aureus* and *S. enterica.*

Most peptides showed hemolytic activity below 30% even at the highest concentration (*Figure 3*). However, peptides La and Ge were hemolytic up to a concentration of 12 µM, while peptide Am was hemolytic up to 1.56 µM.

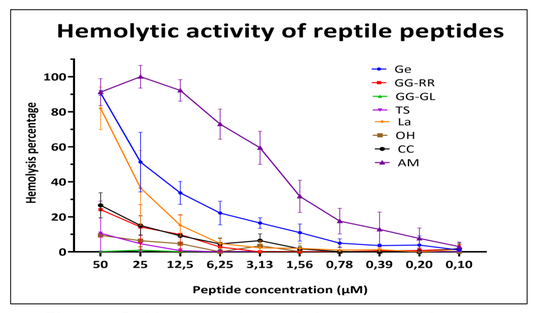
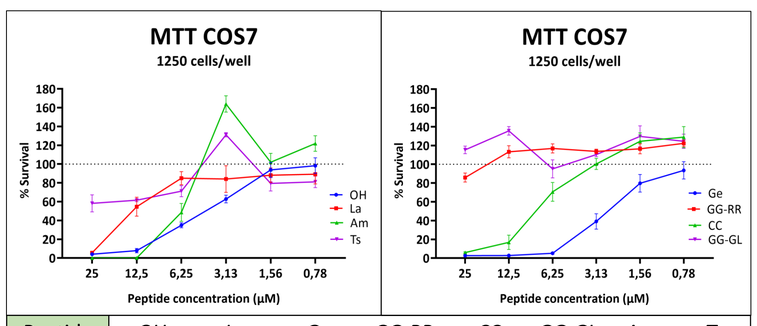


Figure 3. Hemolytic activity of peptides

The results of the MTT assay demonstrate that most of peptides do not exhibit cytotoxicity below a concentration of 6,25 µM (*Figure 4*). However, both Ge peptide and OH reference peptide decrease cell viability down to a concentration of 1,56 µM.



Peptide	*OH*	*La*	*Ge*	*GG-RR*	*CC*	*GG-GL*	*Am*	*Ts*
IC50 (µM)	4.364	12.31	2.594	26.83	8.055	N.A	6.248	27.93

Figure 4. Survival percentage of COS7 cell line after treatment with peptides in MTT assay and IC50 values. N.A means not active.

CONCLUSIONS

Cathelicidin peptides obtained from reptiles showed significant antibacterial activity, while showing minimal cytotoxic and hemolytic activity at their effective concentration. The peptides with the highest activity coincide with those exhibiting greater hydrophobicity, suggesting that this attribute may play a crucial role in determining their attraction to bacterial membranes. Reptile peptides appear to be molecules with potential applications in biomedicine, and it is necessary to continue studying their activity and mechanisms of action. The study of reptilian peptides has revealed their potential utility in the field of biomedicine and therefore warrants further exploration of their activity and underlying mechanisms.

References:
[1]: Alford, et al. (2020). Frontiers in Microbiology, 11. doi: 10.3389/fmicb.2020.01902
[2] van Dijk, et al. (2023). Molecular Immunology, 157, 53–69

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