Functional characterization of crocodylian cathelicidins

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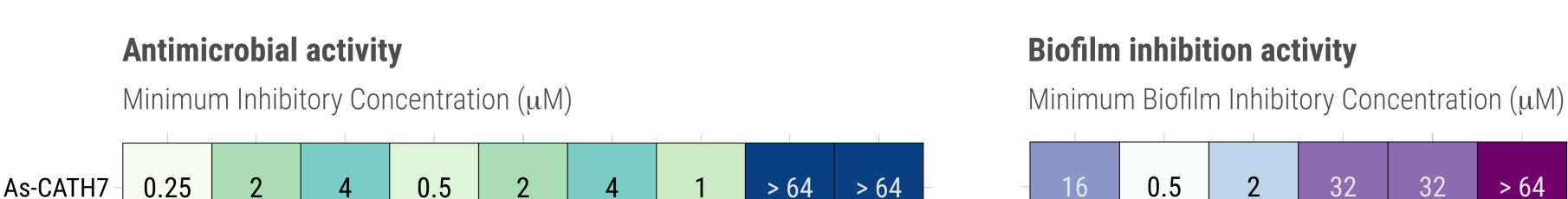
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INTRODUCTION

• Infections caused by antibiotic resistance bacteria (e.g. ESKAPE pathogens) are a major health problem.

• Cathelicidins are one of the major classes of host defense peptides in vertebrates and constitute attractive candidates as novel therapeutics against bacterial infections.
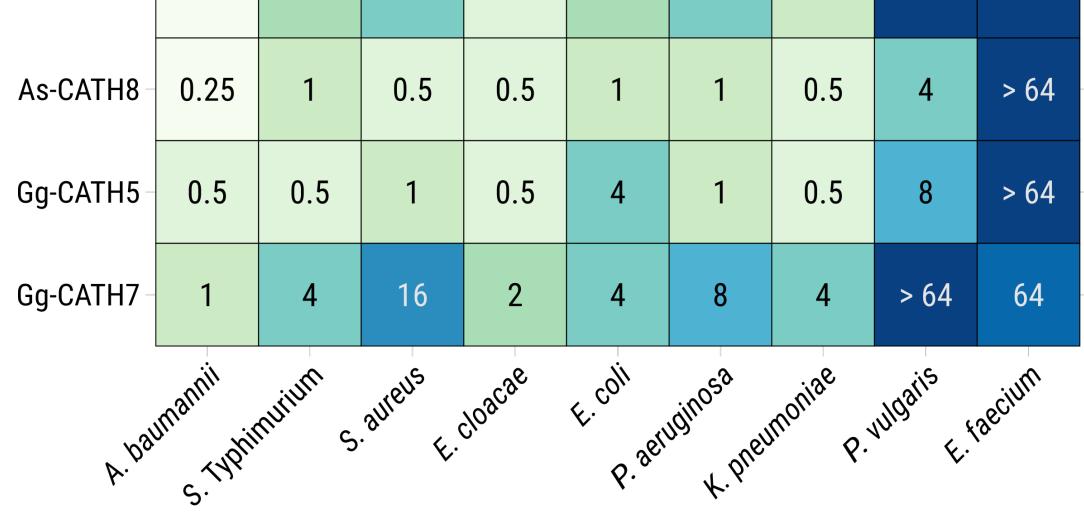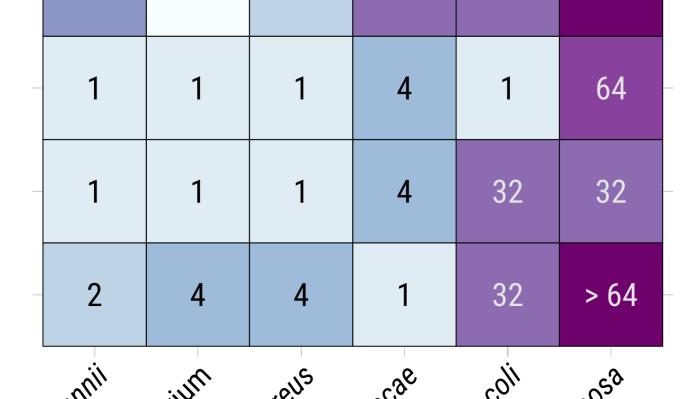
• Crocodylians have a potent immune immune system and are naturally resistant to several pathogens that can affect humans.
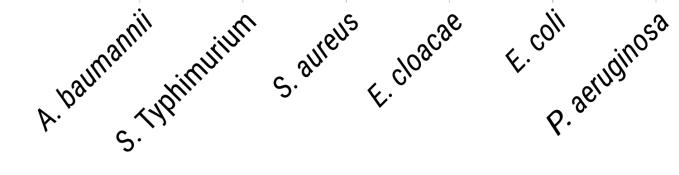
Aim:

To functionally characterize in vitro and in vivo the antibacterial effect of four crocodylian cathelicidins (crocCATHs) against several pathogens relevant to human health.
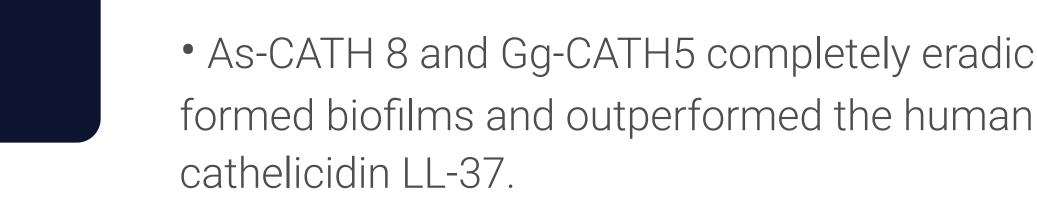
METHODS

1 In vitro antibacterial activity of croCATHs

64   Concentration (μM)   0.25

• Activity against planktonic cells was asssed using the MIC assay.

• Inhibitory activity against biofilms was determined using the crystal violet staining assay.

2 Biofilm eradication in a human skin model

S. aureus

Bacterial Infection (~10^6 CFUs) → 24 hours → Peptide treatment (200 μg) → 24 hours / Bacterial recovery → Plating and CFU count

3 Anti-infective capacity in an abscess model

Bacterium

Bacterial Infection (~10^8 CFU) → 1 hour → Peptide Treatment (15 mg/Kg, intra abscess administration) → 3 days → Abscess tissue collection → Bacterial recovery → Plating and CFU count

CONCLUSION

Crocodylian cathelicidin peptides are promising therapeutic candidates for the treatment of biofilm infections caused by the highly antibiotic resistant pathogens S. aureus and A. baumannii.

RESULTS

CrocCATHs showed broad spectrum in vitro antimicrobial and antibiofilm activities against Gram-positive and Gram-negative bacteria

Antimicrobial activity
Minimum Inhibitory Concentration (μM)

	A. baumannii	S. Typhimurium	S. aureus	E. cloacae	E. coli	P. aeruginosa	K. pneumoniae	P. vulgaris	E. faecium
As-CATH7	0.25	2	4	0.5	2	4	1	> 64	> 64
As-CATH8	0.25	1	0.5	0.5	1	1	0.5	4	> 64
Gg-CATH5	0.5	0.5	1	0.5	4	1	0.5	8	> 64
Gg-CATH7	1	4	16	2	4	8	4	> 64	64

Biofilm inhibition activity
Minimum Biofilm Inhibitory Concentration (μM)

	A. baumannii	S. Typhimurium	S. aureus	E. cloacae	E. coli	P. aeruginosa
As-CATH7	16	0.5	2	32	32	> 64
As-CATH8	1	1	1	4	1	64
Gg-CATH5	1	1	1	4	32	32
Gg-CATH7	2	4	4	1	32	> 64

As-CATH8 and Gg-CATH5 exerted potent eradication capacity against S. aureus in a human organoid model of biofilm infected skin

• As-CATH 8 and Gg-CATH5 completely eradicated pre-formed biofilms and outperformed the human cathelicidin LL-37.
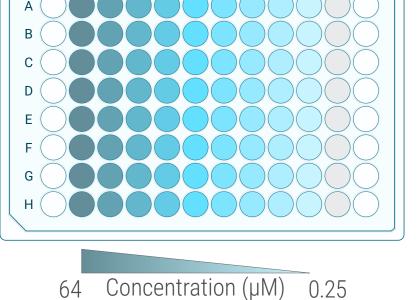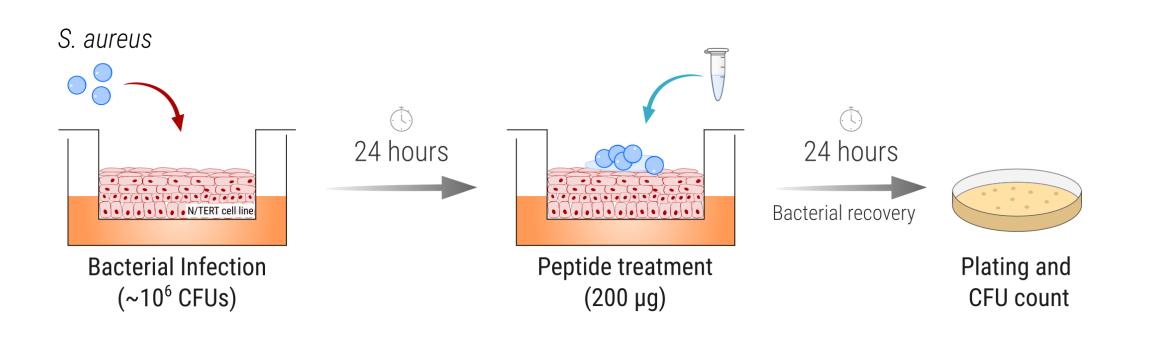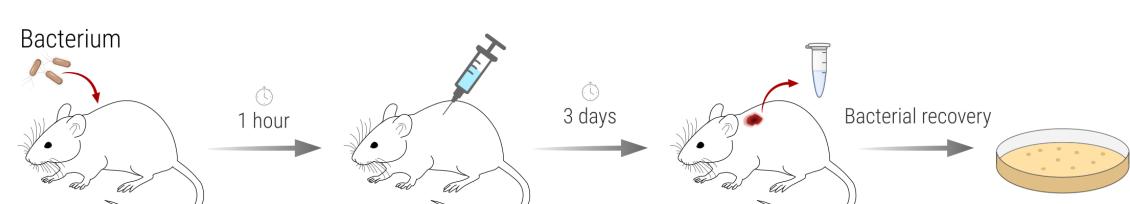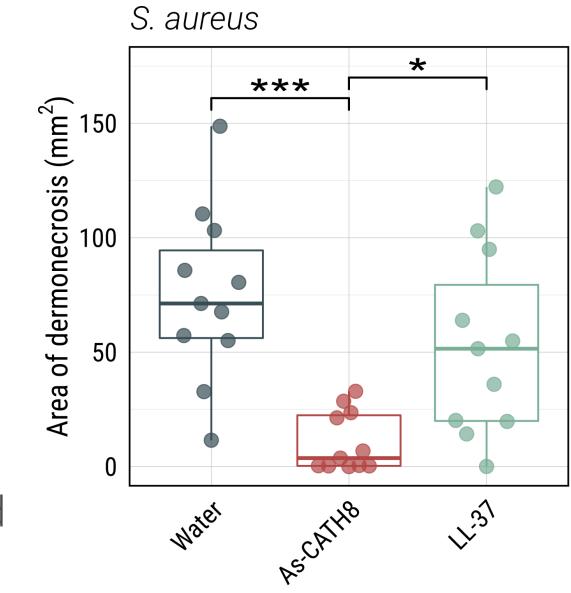
• Kruskal-Wallis test followed by Dunn's posthoc test.

• **: p < 0.01

As-CATH8 displayed strong anti-infective effect in a murine abscess model

• As-CATH8 decreased the area of dermonecrosis and CFU recovered from S. aureus and A. baumannii abscesses.

• Treatment with As-CATH8 showed a stronger effect than the human LL-37.

• Kruskal-Wallis test followed by Dunn's posthoc test or Welch's one-way test followed by Games-Howell posthoc test.
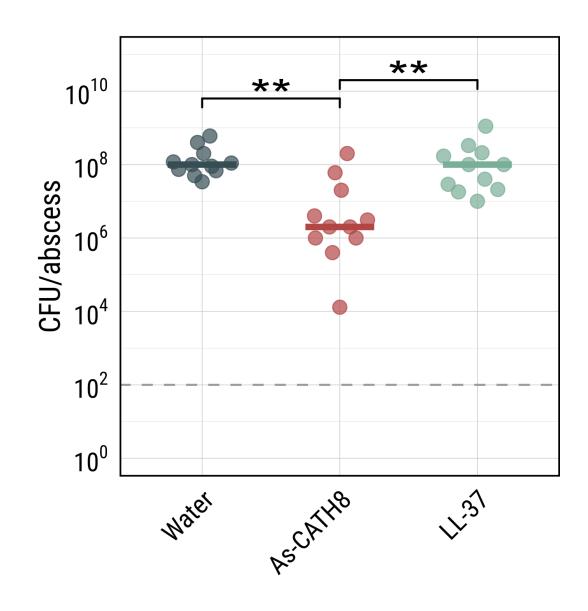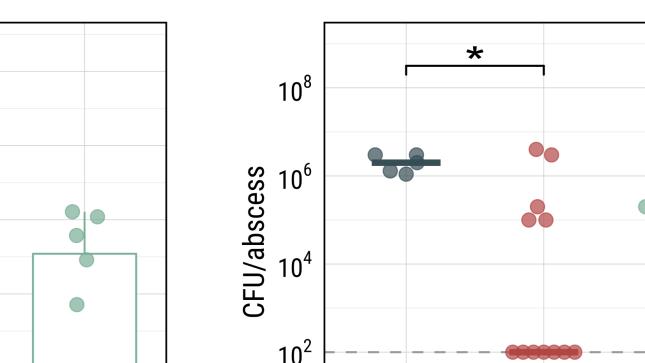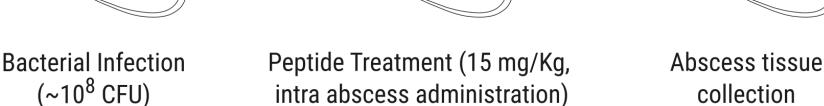
• *, **, ***: p < 0.05, 0.01, 0.001

S. aureus

A. baumannii