Parrots cathelicidin as a new source of antimicrobial agents

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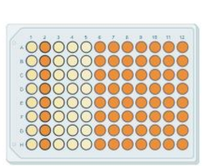
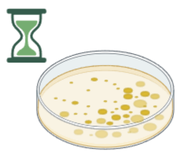
INTRODUCTION

The innate immune system of most vertebrates includes a family of Host Defense Peptides (HDPs) named cathelicidins [1]. Avian cathelicidins are an excellent candidates for antimicrobial drug development due to their spectrum of activity against various microorganisms and low toxicity [2]. Because of their extraordinarily long lifespan, parrots are exposed to a variety of infectious diseases during their lifetime, making them an excellent subject for the study of their immune system. The aim of this study was to evaluate the antimicrobial, hemolytic and cytotoxic activity of parrots cathelicidins and to determine their mode of action.

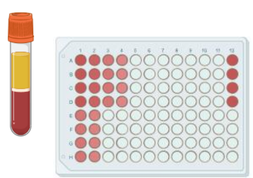
MATHERIAL AND METHODS



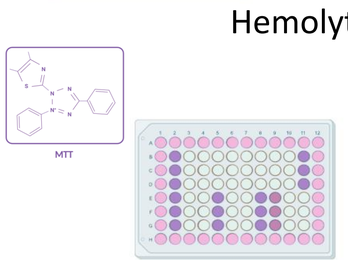
Minimun Inhibitory Concentration
Minimum Bactericidal Concentration



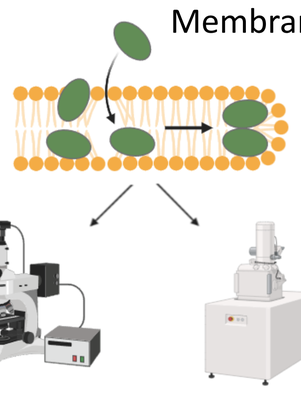
4-hours and 9-hours
Time-kill assay



Hemolytic activity



Cytotoxicity against
VERO cells (MTT)



Membrane disruption

Fluorescence
microscopy
(Sytox green)

Transmission
electron microscopy

RESULTS

Parrots cathelicidins showed a broad-spectrum antimicrobial activity (Fig. 1) with MIC values ranging from 12.5 to 3.125 µM against different human pathogens. In addition, they presented a strong bactericidal activity since MBC was similar to the MIC.

Parrots CATH2s had no negative effect on human erythrocytes and VERO cells, all CATH3s showed hemolytic and cytotoxic effect only at the highest concentrations tested (Fig. 2).

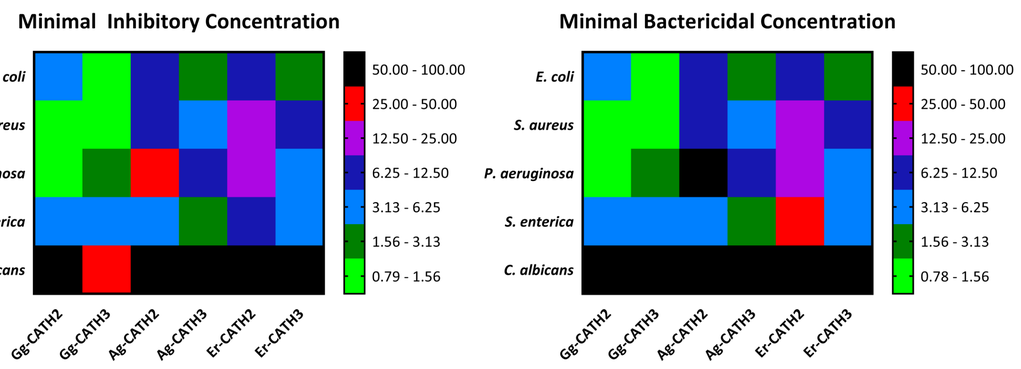


Fig. 1. Minimum Inhibitory and Bactericidal Concentration of avian cathelicidins against different human pathogens. 1x10^5 CFU/mL bacteria were incubated with peptide at different concentration (50 – 0.1 µM) for 18 hours. Samples without visible growth were seed in agar plate after serial dilution for stablish de MIC (same bacterial concentration as in the initial inoculum) and MBC (0.1% of the initial inoculum).

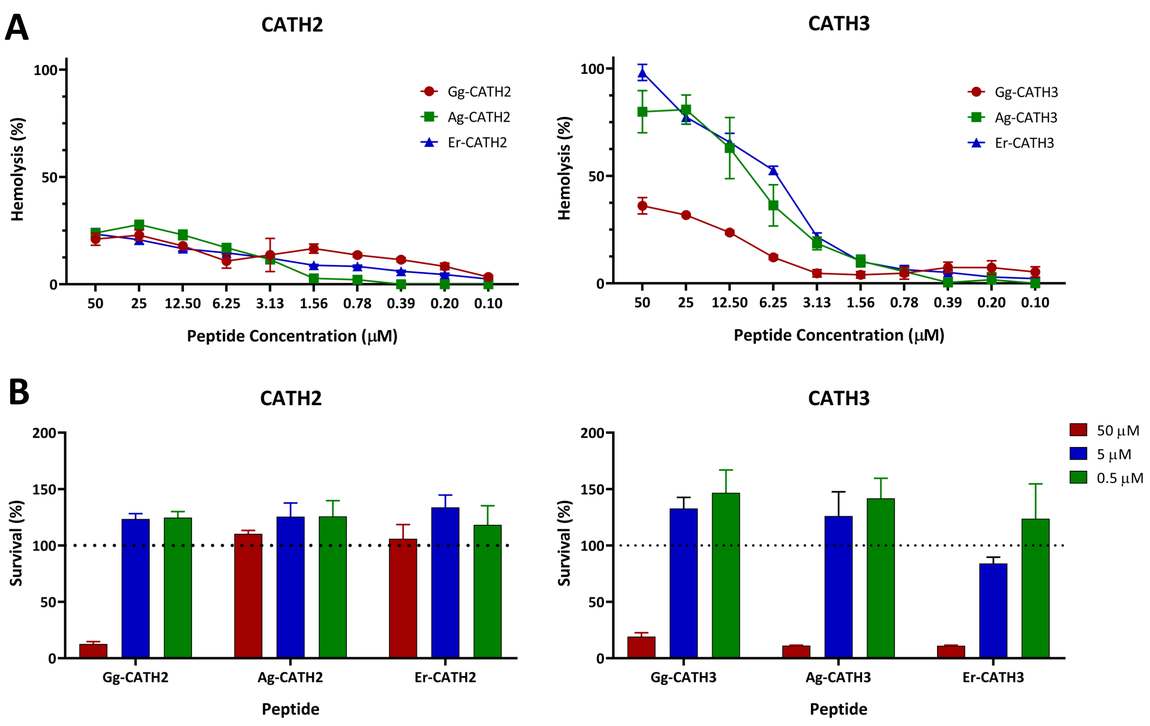


Fig. 2. Toxic activity of avian cathelicidins. Hemolytic activity (A) was studied in human erythrocytes in presence of different concentration of avian cathelicidins. Cytotoxic activity (B) was studied against VERO cells (10000 cells/well) after 18 h of incubation in presence of avian cathelicidins at three different concentrations (50, 5 and 0,5 µM) using the MTT assay.

RESULTS

Ag-CATH3 showed strong bactericidal activity against *E. coli* in latency phase for 9 hours (Fig. 3A). Regarding to *S. aureus*, Ag-CATH3 showed bactericidal effect in latency phase for 4 hours, after that the effect is bacteriostatic as well as in exponential phase (Fig. 3B). Peptide showed sensibility to the inoculum size.

Both parrot CATH2s act faster killing *E. coli* between 10 and 20 mins (Fig. 4A), on the other hand, both parrot CATH3s need between 60 and 120 mins for kill all the bacteria (Fig. 4B).

Sytox green assay showed that all parrot cathelicidins operated by disrupting the bacterial membrane (Fig. 5A). This result was confirmed by electron microscopy (Fig. 5B)

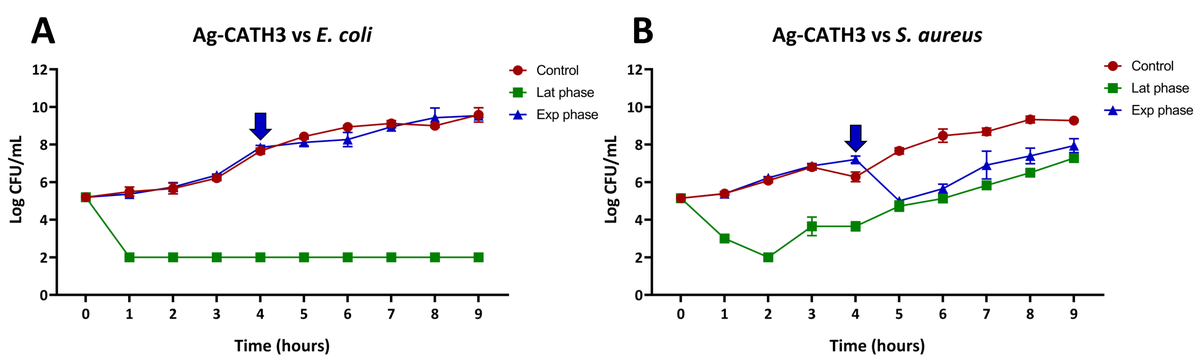


Fig. 3. 9-hours time-kill assay of Ag-CATH3 against *E. coli* (A) and *S. aureus* (B). Bacteria were incubated at 1x10^5 CFU/mL and Ag-CATH3 was added at MIC concentration for both microorganisms (3,125 and 6,25 µM respectively). Blue arrow indicates the hour at which the peptide was added in exponential phase in both curves. Each hour one sample was taken and seeded on agar plates after serial dilutions for colony counting.

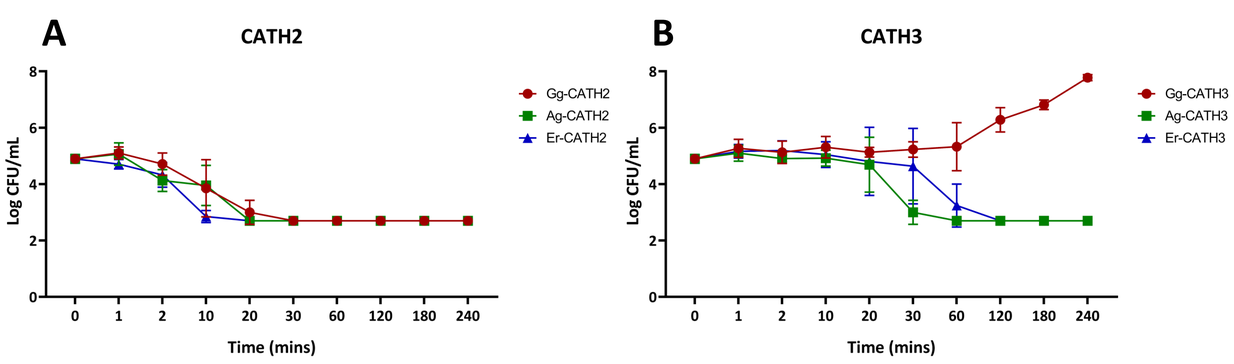


Fig. 4. 4- hours time-kill assay of avian CATH2 (A) and CATH3 (B) against *E. coli*. Bacteria were incubated at 1x10^5 CFU/mL and peptides were added at MIC concentration. In each time point one sample was taken and seeded on agar plates after serial dilutions for colony counting.

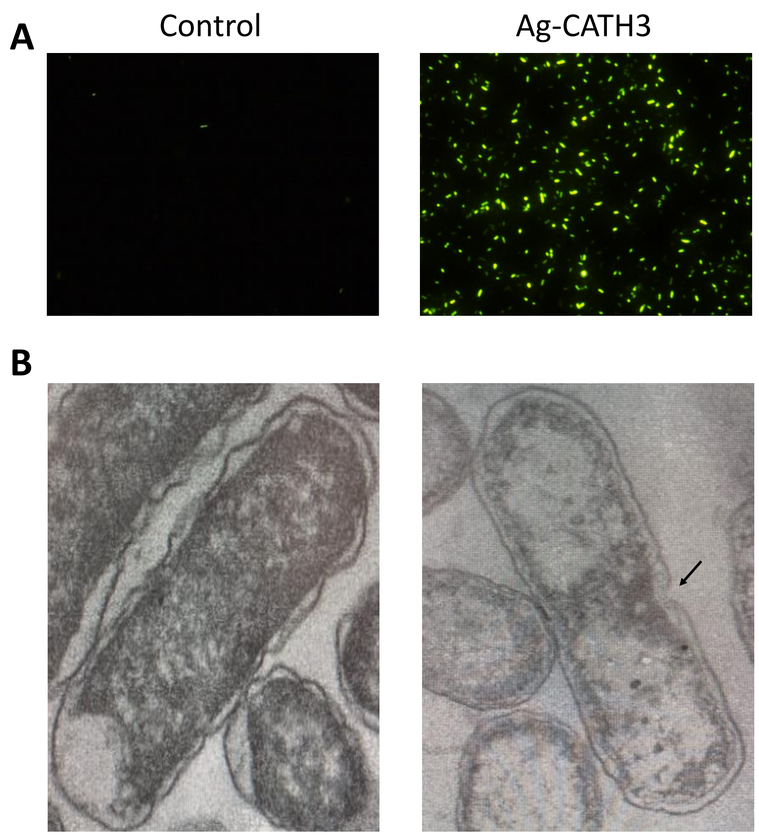


Fig. 5. *E. coli* was incubated with Ag-CATH3 for 30 mins. (A) Fluorescence microscopy using Sytox green. Green colour indicates dead bacteria by membrane disruption (B) Transmission electron microscopy. Black arrow indicate pore formation.

CONCLUSIONS

Parrots cathelicidin-derived peptides showed potent antimicrobial activity and low toxic effects on human erythrocytes and VERO cells. Their mode of action is disrupting the bacterial membrane in a short time. This work showed the potential of parrot cathelicidins to be used as novel templates for antimicrobial drug development.

REFERENCES

1. Alford et al., 2020. DOI: 10.3389/fmicb.2020.01902
2. Xiao et al., 2020. DOI: 10.1016/j.psj.2020.03.021

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