Stability and Activity of the Novel Antimicrobial Peptide Corynacin, a Potential Biopreservative

MOUNT ROYAL UNIVERSITY 1910

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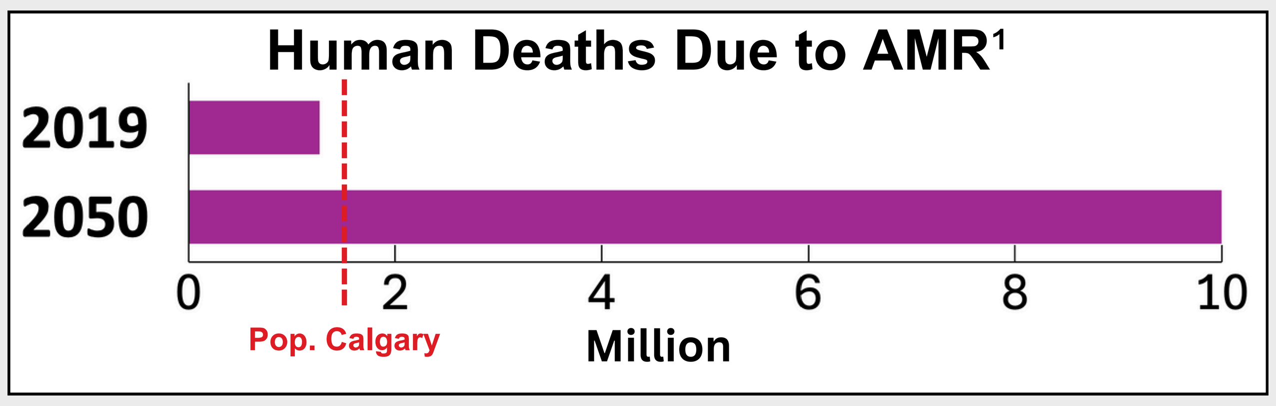
INTRODUCTION

Background

- Misuse of antibiotics has led to an increase in antimicrobial-resistant bacteria (AMR)[1]
- There is an urgent need for alternative treatments
- Bacteriocins show significant potential



Bacteriocins[2]

- Peptides synthesized by bacteria
- Show antimicrobial properties
- Possess the potential to inhibit bacteria that have developed resistance
- Employed as biopreservatives and therapeutic agents

Objective

This research characterizes the bacteriocin Corynacin.

Corynacin

- **Producer:** *Corynebacterium jeikeium*
- **Discovery:** Genome mining
- **Length:** 52 Amino acids
- **Mass:** 5,849 Daltons
- **Class:** Leaderless (IID)

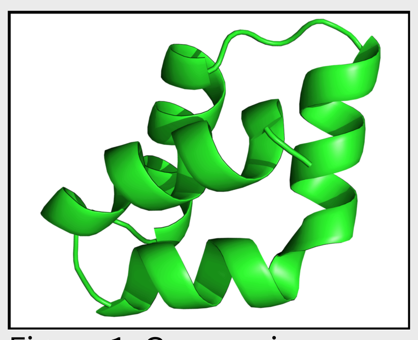


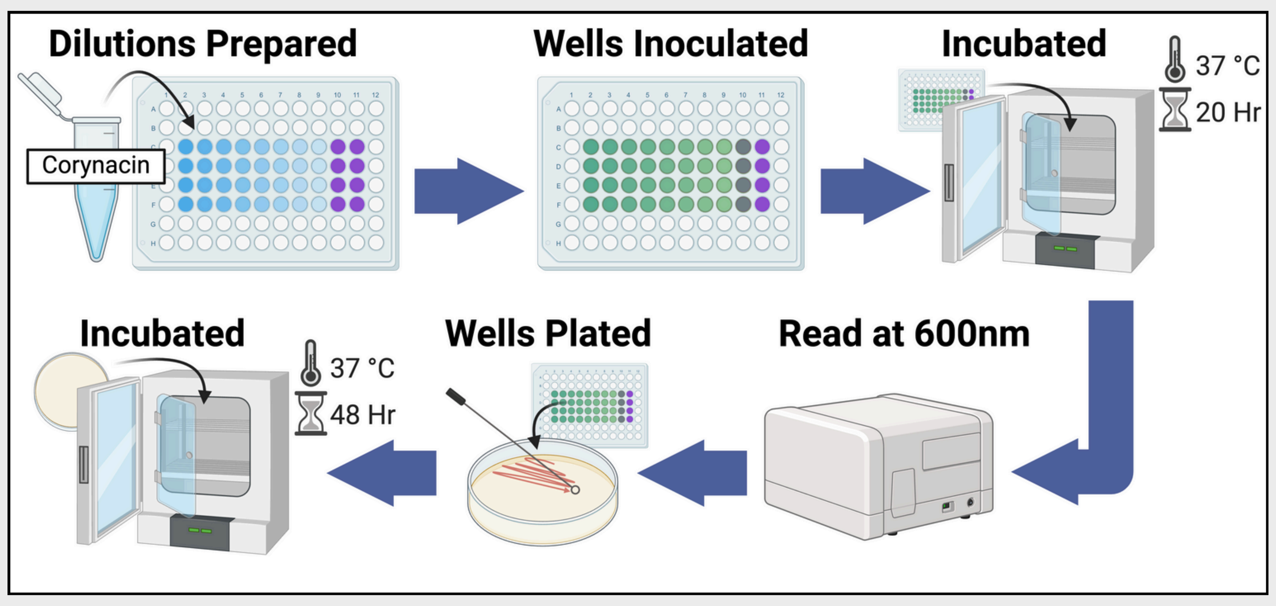
Figure 1. Corynacin structure predicted using AlphaFold 3.

RESULTS

Corynacin demonstrates

- **Broad activity**
  - Exhibits antimicrobial action against close and distantly related bacteria
- **High stability**
  - Temperature 0°C to 100°C
  - pH 2 to pH 8
  - Salt concentration 0% to 7%
  - Proteases Trypsin, Chymotrypsin, and Proteinase-K
- **Pore-forming mode of action**
  - Leakage of potassium and magnesium ions
  - Macromolecule leakage of DNA

ACTIVITY



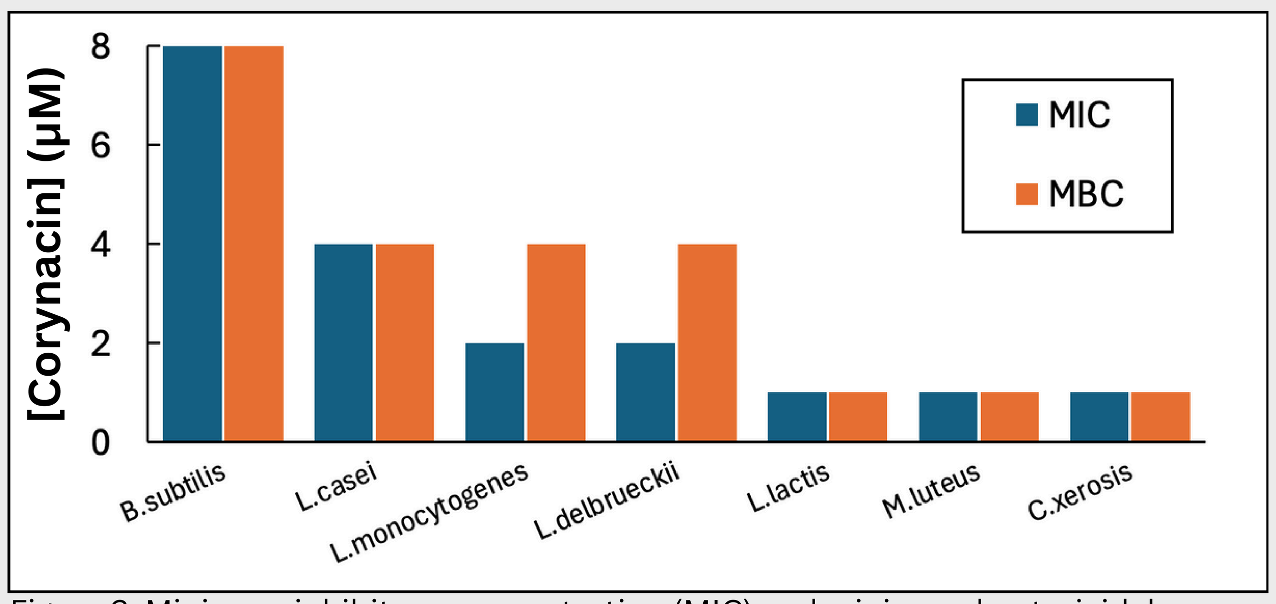
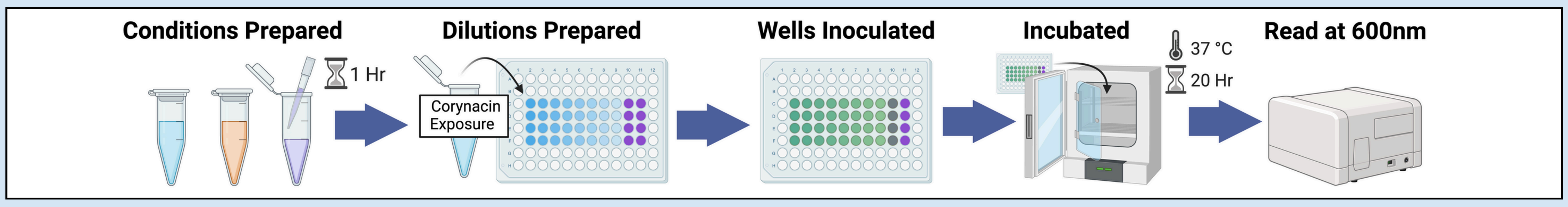
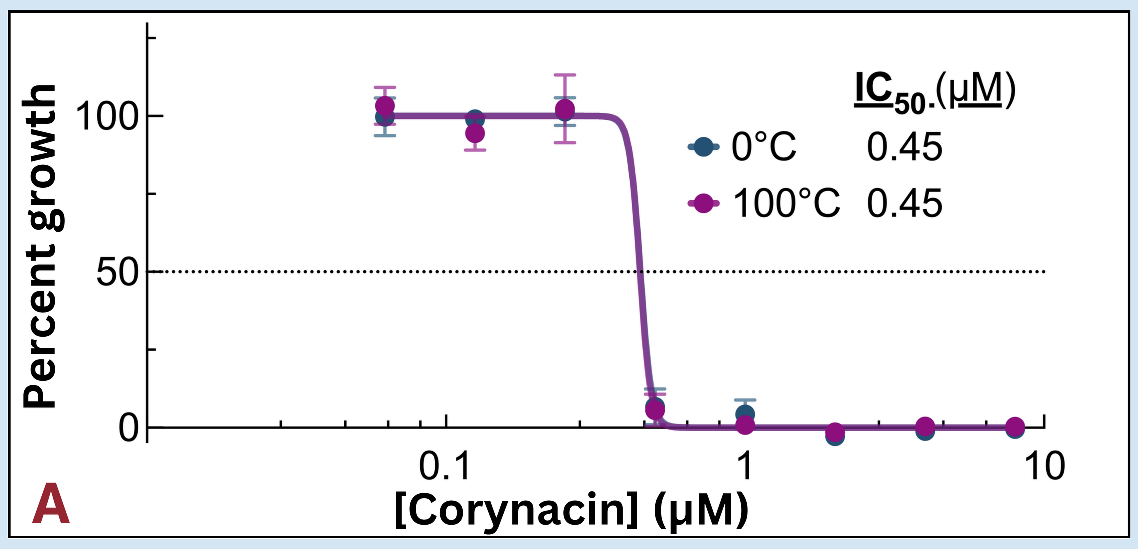
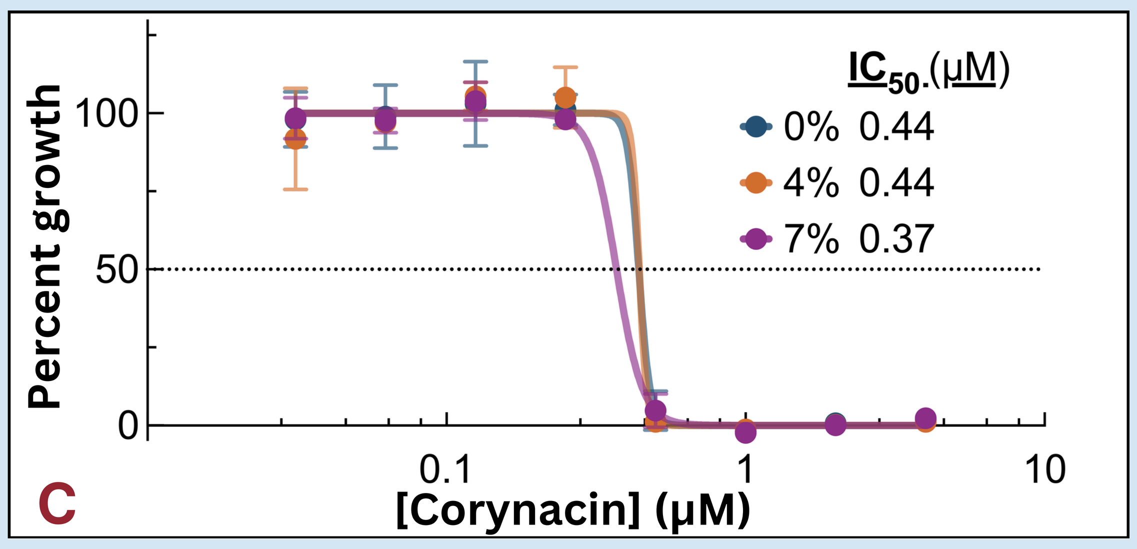


Figure 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Corynacin with respect to various bacteria determined using broth microdilution assay.

STABILITY







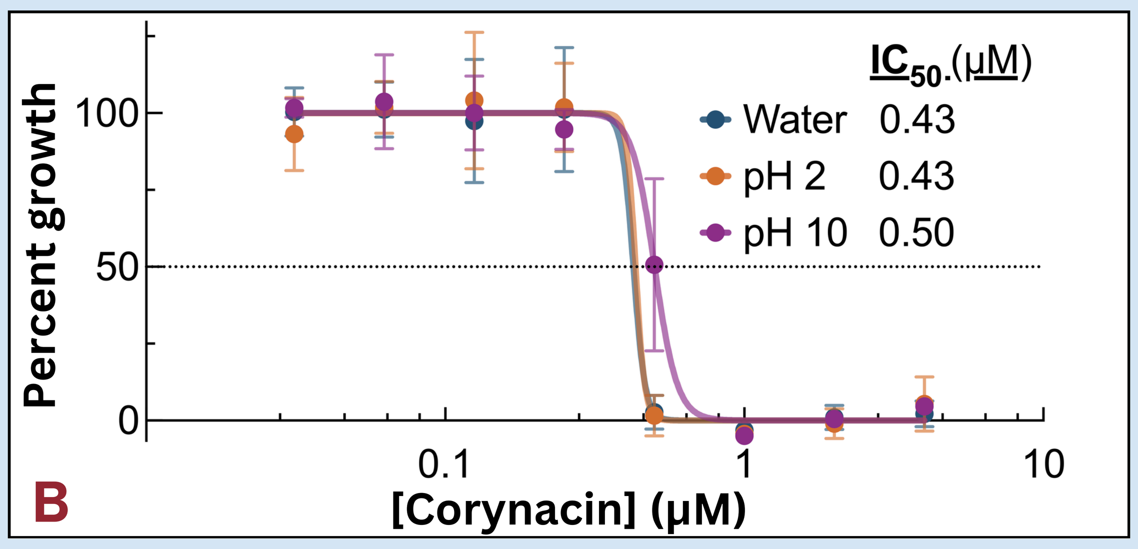
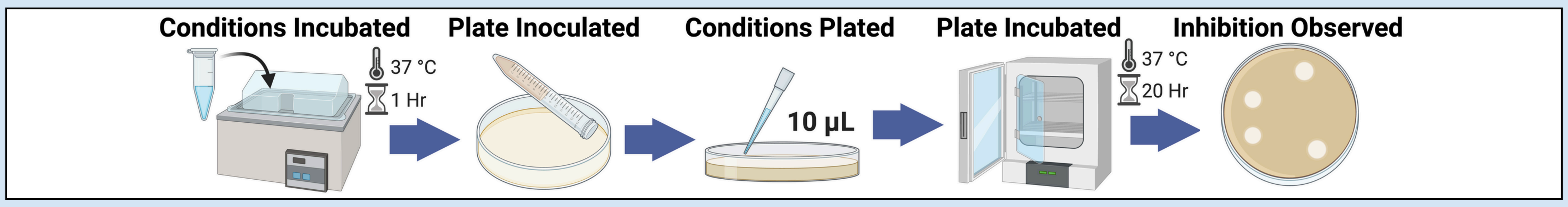


Figure 3. Normalized *L. lactis* growth when treated with Corynacin exposed to various conditions. (A) Thermal exposure of 100°C relative to 0°C control. (B) 4% and 7% salt exposure relative to 0% control. (C) pH 2 and pH 10 buffer exposure relative to a water control. Dose response curve and IC_{50} were calculated using GraphPad Prism 10.4.1.

The Half Maximal Inhibitory Concentration (IC_{50}) is the concentration of Corynacin required to inhibit 50% of bacterial growth. This metric provides enhanced sensitivity in evaluating changes in inhibitory activity.



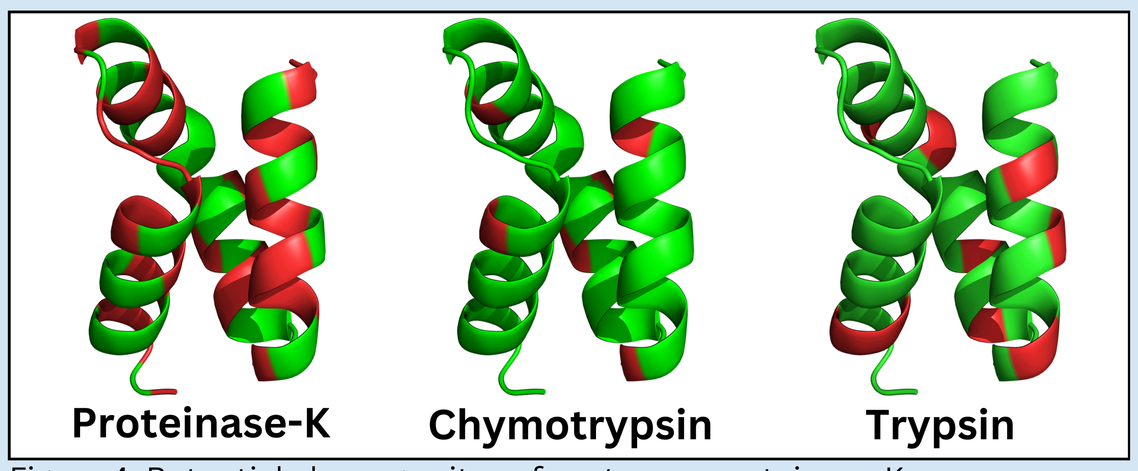


Figure 4. Potential cleavage sites of proteases proteinase-K, chymotrypsin, and trypsin, are indicated in red. Corynacin structure was predicted using AlphaFold 3. Images were created using Pymol 3.1.4.

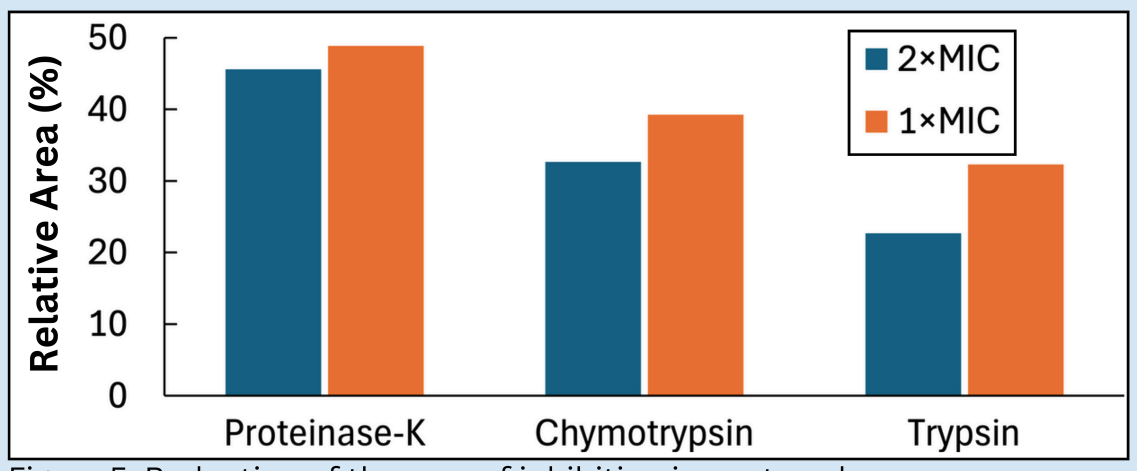
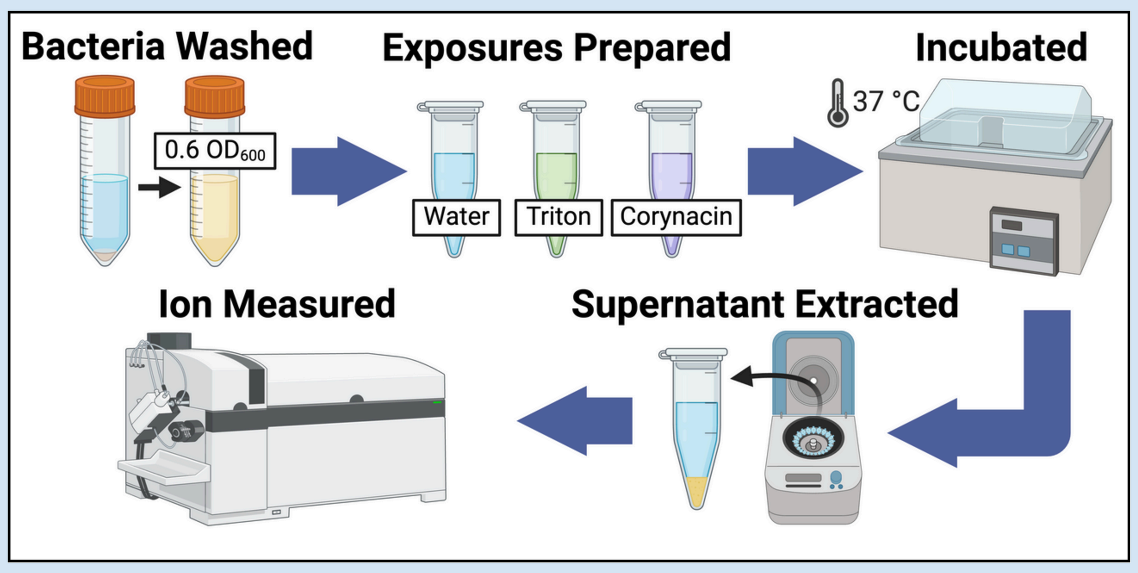
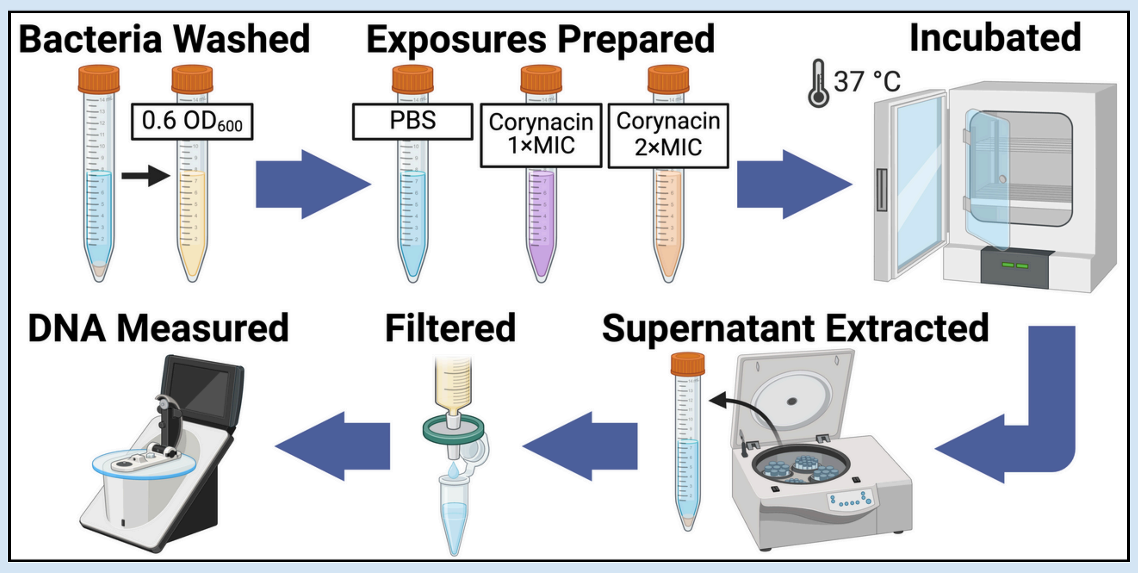
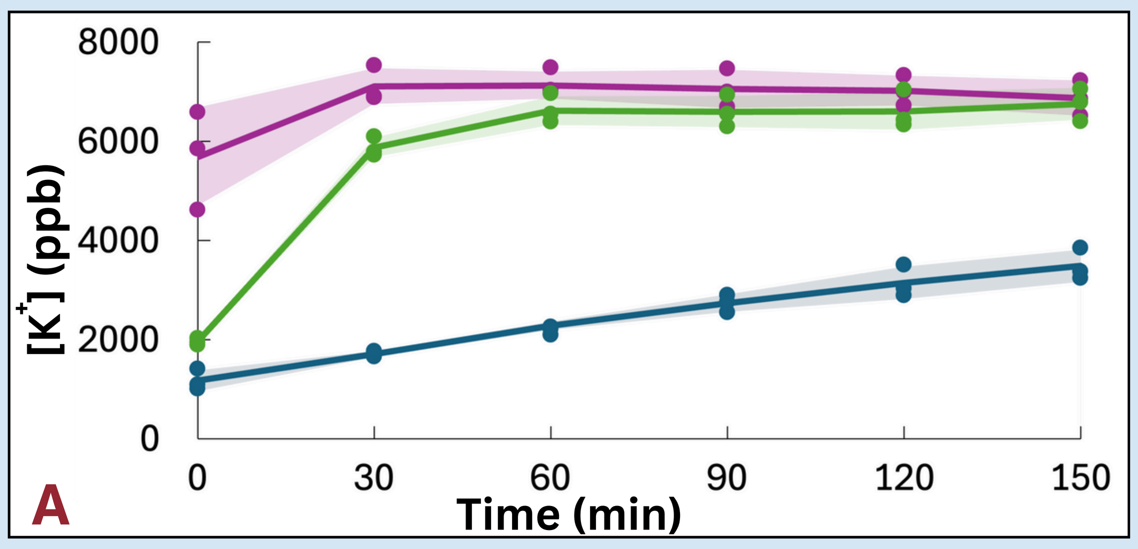


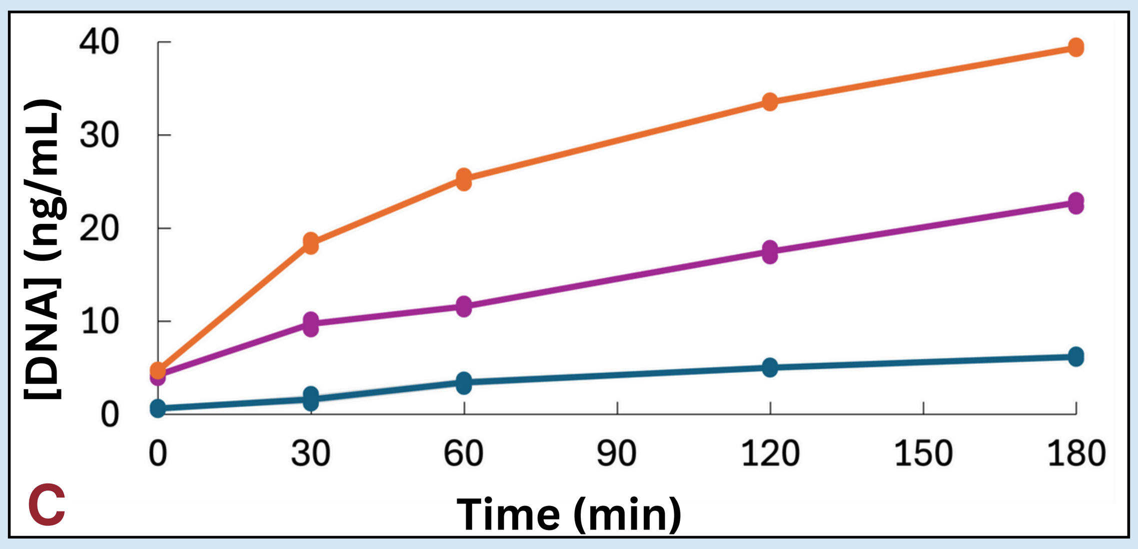
Figure 5. Reduction of the area of inhibition in spot-on-lawn assay following Corynacin exposure to various proteases.

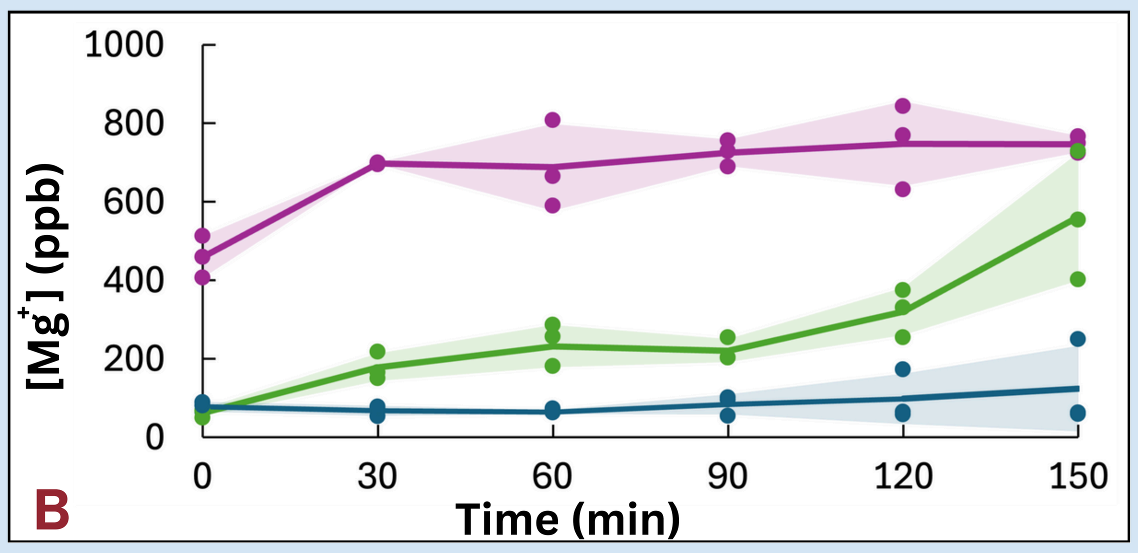
MODE OF ACTION











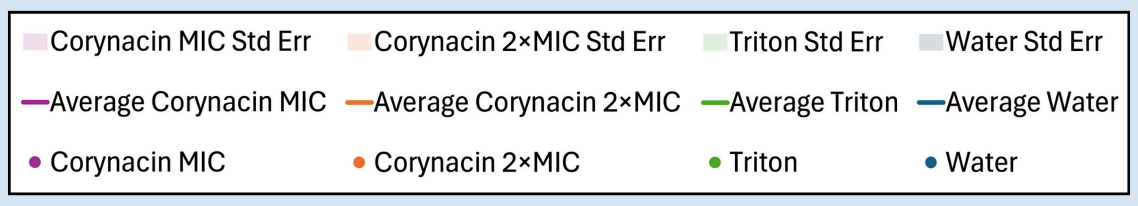


Figure 6. Leakage of intercellular contents of *L. lactis* due to various exposures. (A) Leakage of intercellular potassium after exposure to the Corynacin MIC (1μM), 1% Triton X-100 or a water blank. (B) Leakage of intercellular magnesium after exposure to identical conditions to 'A'. (C) Leakage of DNA after exposure to the Corynacin MIC (1μM), Corynacin 2×MIC (2μM) or a water blank.

CONCLUSION

The stability and activity of Corynacin indicate it is a strong candidate for use as a biopreservative. Additionally, Corynacin has demonstrated pore formation, a distinct mode of action relative to traditional antibiotics. This mode of action indicates Corynacin may exhibit activity against bacterial strains with AMR[2]. Future research will characterize the protease stability of Corynacin quantitatively using HPLC and hemolytic activity in human blood.

References

(1) Naghavi, M.; Vollset, S. E.; Ikuta, K. S.; Swetschinski, L. R.; Gray, A. P.; Wool, E. E.; Aguilar, G. R.; Mestrovic, T.; Smith, G.; Han, C.; Hsu, R. L.; Chalek, J.; Araki, D. T.; Chung, E.; Raggi, C... Global Burden of Bacterial Antimicrobial Resistance 1990–2021: A Systematic Analysis with Forecasts to 2050.

(2) The Lancet 2024, 404 (10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1. Soltani, S.; Hammami, R.; Cotter, P. D.; Rebuffat, S.; Said, L. B.; Gaudreau, H.; Bédard, F.; Biron, E.; Drider, D.; Fliss, I. Bacteriocins as a New Generation of Antimicrobials: Toxicity Aspects and Regulations. FEMS Microbiology Reviews 2021, 45 (1), fuaa039. https://doi.org/10.1093/femsre/fuaa039.

Acknowledgements

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AMR One Health Consortium

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