Underrepresented Sequence–Derived Peptides as a Novel Antimicrobial Platform to Mitigate Resistance Development

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INTRODUCTION

Antimicrobial resistance is a major global challenge, driving the need for novel antibacterial strategies. A key limitation in antibacterial activity is membrane permeability, which affects the uptake and efficacy of antimicrobial compounds[1].

A previous study from our lab[2] developed an in-house computational approach to analyze whole-organism proteomes and identify short (3–4 amino acids) linear sequences that are significantly underrepresented (URS). These sequences were found to be species-specific and, in some cases, completely absent from bacterial proteomes. It was proposed that their absence results from a deleterious effect on cell viability, a hypothesis that was supported by experimental validation.

Since similar sequences are not underrepresented in the human proteome, URSs may serve as a basis for developing novel antibiotics. We show that synthetic peptides containing URS (URSpep) exhibit bactericidal properties when incubated with bacterial cells. In addition, recent structural determination of ribosomes bound to URSpep provides insight into their molecular interactions with the ribosome (Tarabeh et al., in preparation).

Our current work focuses on improving peptide permeability and stability to optimize intracellular targeting and enhance antibacterial activity.

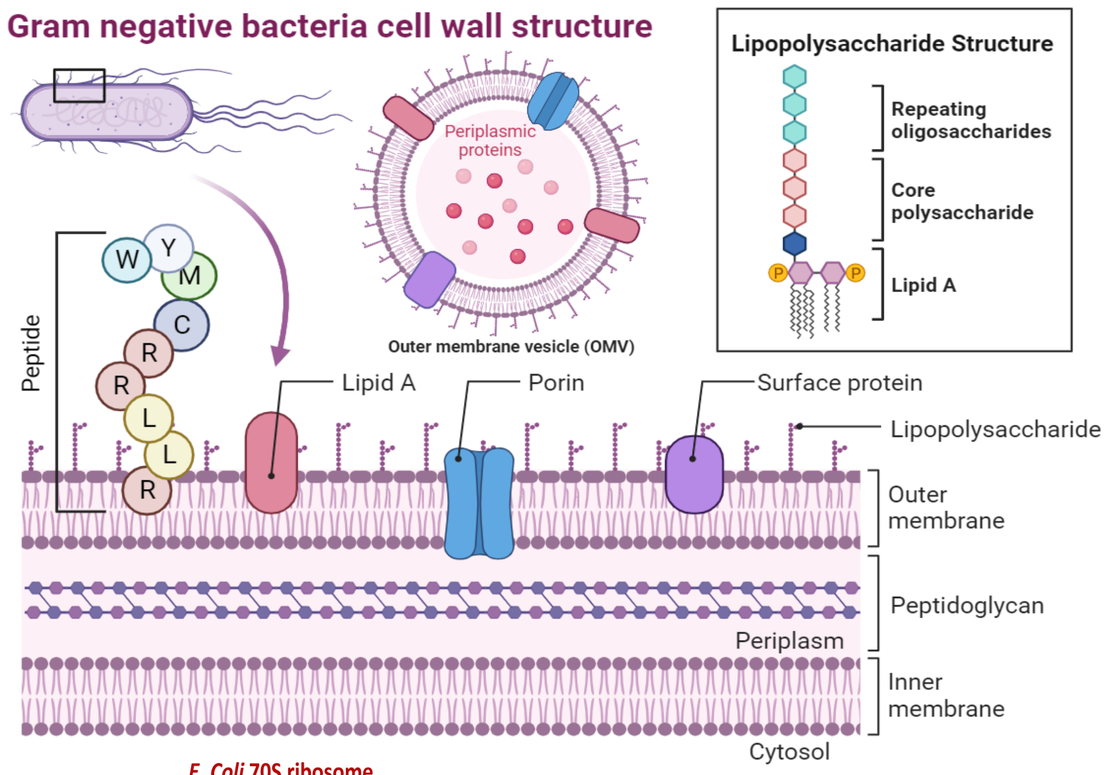


Fig.1.

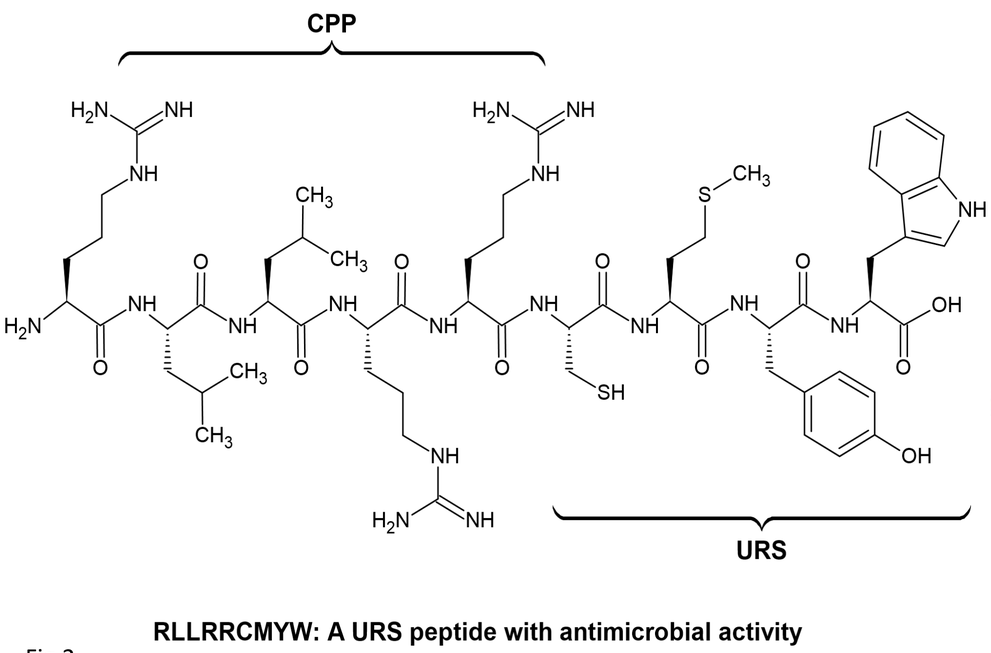


Fig.2.

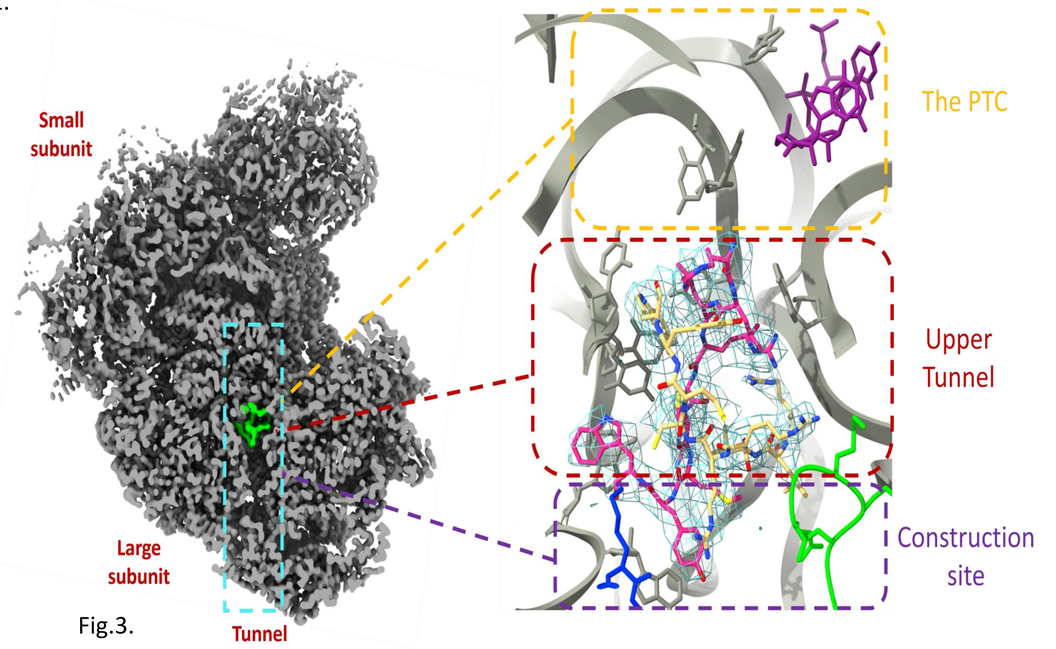


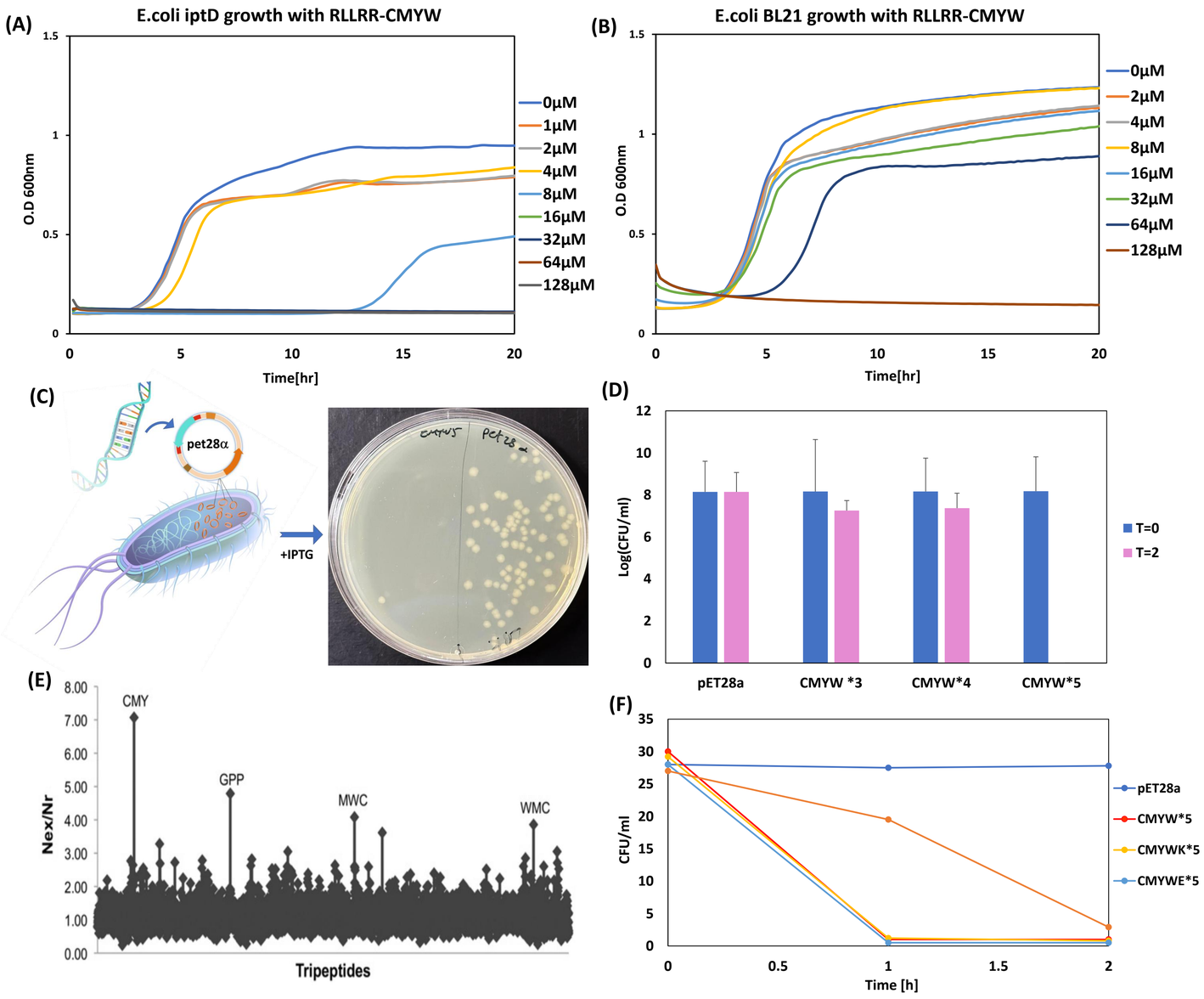
Fig.3.

RESEARCH GOALS

The main goal of this study is to characterize the physicochemical properties of URS-containing peptides (URSpep) in relation to their antibacterial efficacy (MIC), cellular penetration, peptide stability, and potential to prevent resistance. Based on these insights, we aim to design improved URSpep with enhanced antibacterial activity.

We further propose that the availability of multiple URSpep targeting diverse pathogenic bacteria may provide a strategy to overcome the development of resistance.

RESULTS



Analysis of *E. coli* growth kinetics and gene expression in the presence of URS sequences.
(A–B) Growth curves of *BL21 WT* and *lptD* (a membrane-permeable mutant) following incubation with increasing concentrations of the peptide.(C) Gene expression comparison between pET-28a(+) plasmid and URS-expressing cells following IPTG induction.(D) Effect of URS repeat copy number on gene expression from plasmid-based URS variants at 1 and 2 hours post-IPTG induction.(E) Identification of *E. coli* triplet URSs by proteomic analysis [2].
(F) Gene expression comparison across plasmids carrying different URS variants, measured 2 hours after IPTG induction.

METHODS

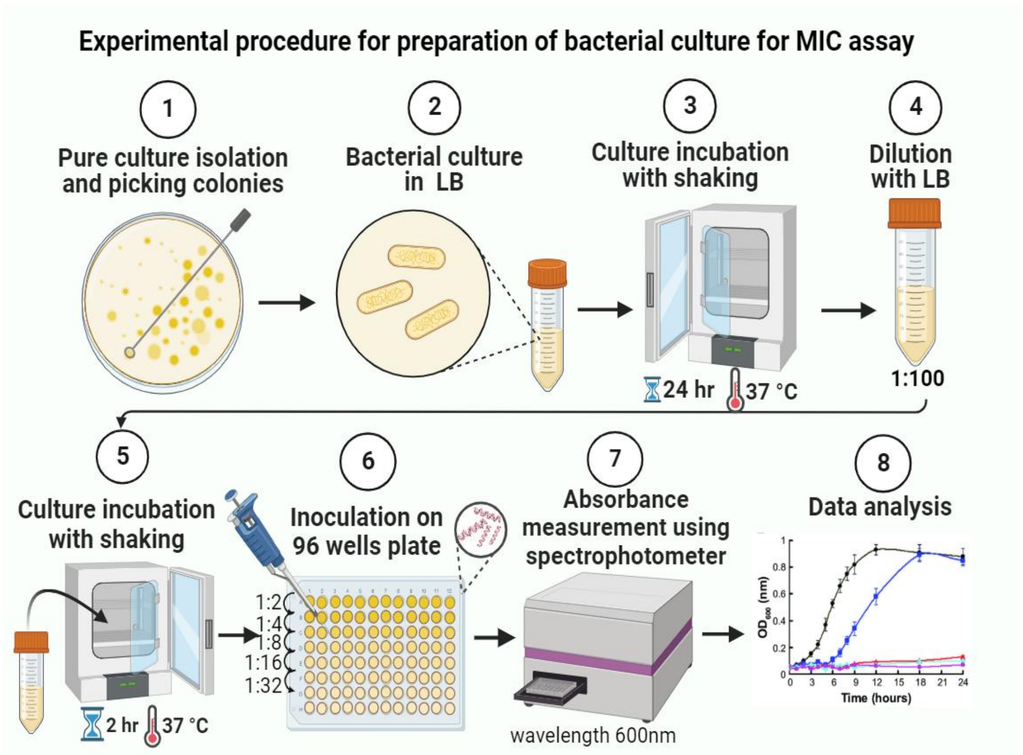


Fig.4.

FUTURE PLANS

Future plans include utilizing messenger RNA (mRNA) encoding URSpep to overcome membrane permeability limitations, examining bacterial resistance to URSpep, and determining the structure of the ribosome bound to improved URSpep using cryo-EM. In the longer term, following extensive characterization, URSpep will be evaluated in more complex models such as cell cultures and animal models.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Ruiz, N., Kahne, D. & Silhavy, T. J. Advances in understanding bacterial outer-membrane biogenesis. *Nat. Rev. Microbiol.* **4**, 57–66 (2006) .

[2] Navon SP, Kornberg G, Chen J, Schwartzman T, Tsai A, Puglisi EV, Puglisi JD, Adir N. Amino acid sequence repertoire of the bacterial proteome and the occurrence of untranslatable sequences . Proc Natl Acad Sci U S A. 2016 Jun 28;113(26):7166-70. doi: 10.1073