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Bioassays of Analogs of Pleurocidin-Amide Indicate that Change at *N*-Terminus is Key to Improved Efficacy

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Introduction

We have a problem. Many microbial pathogens are becoming resistant to conventional therapeutics, and are no longer affected by them. We need to develop the next generation of antimicrobials or return to times when a minor bacterial infection could be fatal. Antimicrobial peptides (AMPs) typically have broad-spectrum specificity, which makes them ideal for critical treatment situations when the identity of the pathogen is unknown. Pleurocidin, an AMP found in secretions from winter flounder (*Pleuronectes americanus*), adopts an amphipathic α-helical structure crucial for activity, and this conformation is induced or stabilized by peptide-membrane interactions [1]. We evaluated the impacts of novel pleurocidin analogs on the exponential growth curves of two Gram (+) and two Gram (-) human pathogens using high-throughput broth bioassays capable of simultaneous monitoring the growth of multiple bacterial species on a single 96-well plate [2]. Our hypothesis holds that specific additions to the *N*-terminus of our "base peptide" pleurocidin-amide, (**LM4-12**), which were designed to enhance initiation of peptide-cell membrane interactions, will increase antimicrobial efficacy and selectivity [3]. We have tested the effects of grafting combinations of **R**, **G**, & **W** onto the *N*-terminus of base peptide **LM4-12**, synthesized using Boc-SPPS methods on an MBHA resin [4]. Comparison of the effects of adding each of these three amino acids to the *N*-terminus of **LM4-12** generated the lead peptide **LM4-15**, with an *N*-terminal arginine, that yielded dramatically improved broad-spectrum efficacy against *Enterococcus faecalis* (EF), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA) and *Escherichia coli* (EC).

LM4-15 (*N*-Ter) H-RGWGSFFKKAAHVGKHVGKAALTHYL-NH_2 (*C*-Ter)

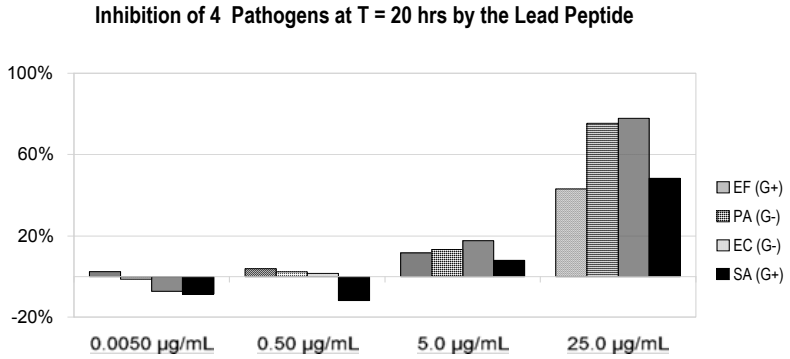

*Fig. 1. Lead Peptide **LM4-15**.*

Results and Discussion

Lead peptide **LM4-15** (Figure 1) showed a significant efficacy against all four human pathogens, with ≥48% growth inhibition relative to untreated bacterial cultures at the maximum tested dose (25 µg/mL, 8.72 µM). Base peptide **LM4-12** (Figure 2) showed a similar inhibitory effect, but only on two of the four pathogens (both Gram +), and some species selectivity, with the highest efficacy against SA. Growth inhibition was calculated as the OD_{630} of peptide-treated bacteria, divided by the OD_{630} of identical untreated cells grown in broth cultures, under the same conditions, measured 20 hours after a single treatment with the test peptide at the indicated dose.

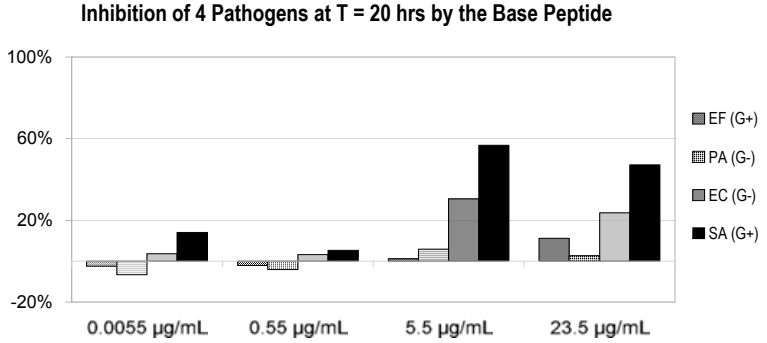

*Fig. 2. Base Peptide **LM4-12**.*

MIC assays typically test the effectiveness of potential antibiotics using a fairly low (10^4 CFU) inoculum of bacteria. Our data, obtained at a higher starting concentration of bacteria (OD_{630} = 0.12 at t = 0), shows that **LM4-12** reached a maximum efficacy for SA, and EC at the second highest dose of the base peptide tested (5.5 µg/mL, 1.85 µM), but was not effective against either PA or EF, even at the highest tested dose (23.5 µg/mL, 8.64 µM). **LM4-15** however, was found to reach a much higher efficacy against all 4 target species at the maximum tested dose of the lead peptide (25.0 µg/mL, 8.72 µM), attaining an efficacy of greater than 48% growth inhibition when tested on gram positive microorganisms SA and EF. What is more, **LM4-15,** at the highest tested dose, showed greater than 73% growth inhibition when tested on the gram negatives, EC and PA, which were not inhibited by **LM4-12** to any great extent (Figures 3-6).

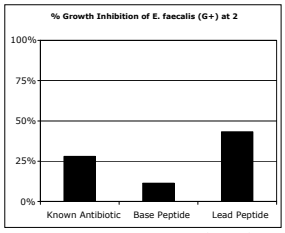

Fig. 3. EF (G+).

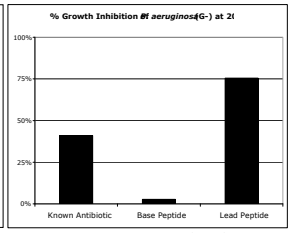

Fig. 4.PA (G-).

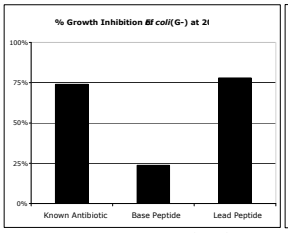

Fig. 5. EC (G-).

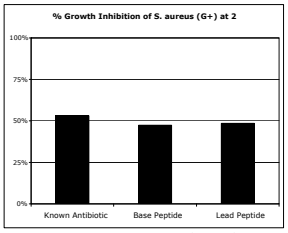

Fig. 6. SA (G+).

In conclusion, modifying the *N*-terminus of the base antimicrobial peptide by adding arginine dramatically enhanced antimicrobial efficacy in the lead peptide **LM4-15** over that of the base peptide **LM4-12**. As the assay data in Figures 1 and 2 demonstrates, peptide doses as low as 5 µg/mL slightly retard bacterial growth, with a significant level of inhibition reached at only 25 µg/mL, the maximum dose assayed in these experiments. Comparing the efficacies of the lead and base peptides with those of a known antibiotic, oligomycin, confirmed that the lead peptide inhibited bacterial growth of three of the four pathogenic microbial species tested (EF, PA, and EC) as well as (or better than) the known antibiotic in our assays. This lead peptide shows great promise as a therapeutic agent, especially now that more bacteria are becoming increasingly resistant to conventional antibiotics. The dramatic increase in antibiotic efficacy observed with a single arginine addition onto the *N*-terminus of pleurocidin in **LM4-15**, versus the low efficacy of similar synthetic peptide analogs prepared with tryptophan, lysine, and glycine at that position, suggests that *N*-terminal arginine addition onto existing AMPs may prove to be a general strategy for increasing AMP effectiveness in targeting diverse bacterial membranes.

References

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