Human microbiome-derived synthetic peptides with antibacterial activity

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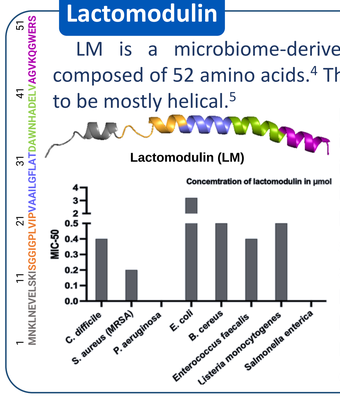
Introduction

The emergence of multidrug resistant (MDR) pathogens or "superbugs" and antimicrobial resistance (AMR) is an ever-increasing concern to global health claiming millions of deaths worldwide.[1] A sharp decline in antibiotic development since the 1980s contrasts with the urgency of developing novel drugs with diverse modes of action against critical pathogens. Peptides display a wide range of bioactivities and desirable therapeutic and pharmacological features.[2] Most notably, antimicrobial peptides (AMPs) have emerged as potential candidates to combat these pathogens. AMPs are found in all kinds of life and are used as the first line of defense against pathogens.[3] They usually have a rapid onset of activity against a wide range of pathogens and a very low prevalence towards resistance development. AMPs are also known to have a dual antibiotic and anti-inflammatory activity, as they can either directly kill bacteria through cell membrane lysis or indirectly through immunomodulation of the host. In this study, we explore the optimization of a natural AMP, lactomodulin (LM), as a potential precursor of antibiotics.

Lactomodulin

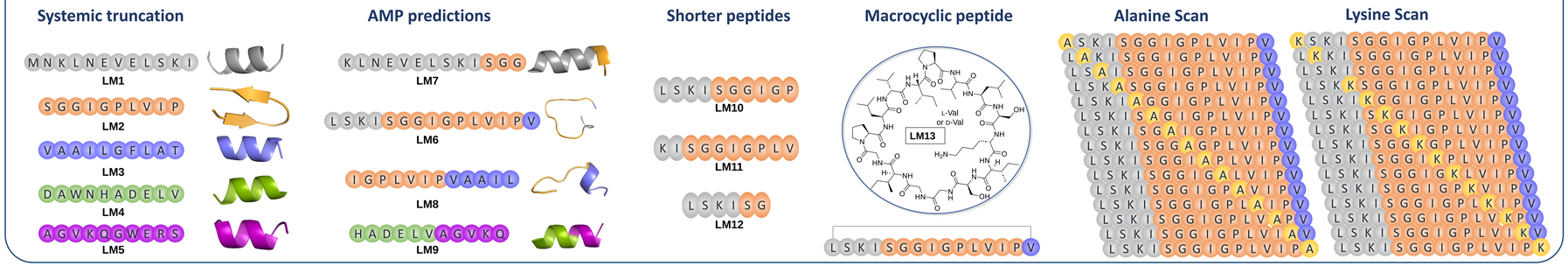
LM is a microbiome-derived bacteriocin AMP from *L. rhamnosus* composed of 52 amino acids.[4] The structure of LM is predicted by AlphaFold to be mostly helical.[5]



LM has been found to have potent anti-inflammatory effect by suppressing pro-inflammatory cytokines. More importantly, it is active against a range of Gram-positive bacterial pathogens including drug-resistant strains such as MRSA and VRE. However, its antibacterial mode of action is not yet known, but is suspected to target the bacterial membrane as most AMPs.[6] Interestingly, LM has shown minimal cytotoxicity against two colon cell lines.

Peptide Design

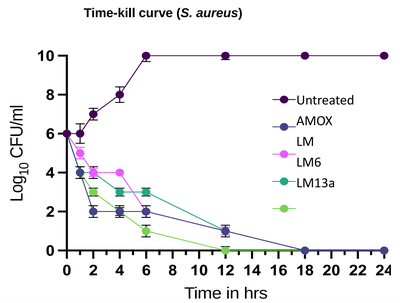
A series of truncated derivatives of varying lengths (10-15) were generated. First, a systemic truncation resulted in five peptides. The second relied on AMP predictions as determined by CAMPR3 using four different algorithms.[7] Secondary structures was predicted by PEP-FOLD3.[8] Based on bioactivity results, further shorter derivatives were tested, as well as the cyclic version of LM6. Additionally, an alanine scan was conducted on the best sequence LM6 to guide further modifications.

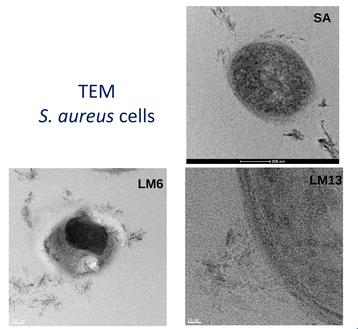


Bioactivity

MIC (µM)

	LM	LM2	LM5	LM6	LM9	LM13	LM10	LM11	LM12	LM-A3	LM-A4	LM-A5	LM-A6
S. Aureus	0.8	2.1	1.2	0.9	1.3	0.8	2.4	5.1	3.9	2.5	3.8	3.2	4.9
MRSA	1.4	-	1.4	1.7	2.5	1.7	-	-	-	3.5	4.3	4.8	5.5
C. Difficile	1.1	1.6	2.1	0.9	1.0	0.9	4.1	-	4.6	3.1	3.1	3.1	5.0
VRE	1.2	-	1.9	1.3	3.1	1.5	-	-	-	-	-	-	-
E.coli	7.5	8.9	7.8	9.2	-	8.2	-	-	-				
P. aeruginosa	-	9.5	-	9.3	-	8.3	-	-	-				





Summary

We determined the key core regions of the LM peptide with antibacterial action. We further explored the best hit peptide, **LM6**, with the generation of shorter derivatives that were less active. However, macrocyclization proved successful showing improved activity. Preliminary studies into the mode of action show a rapid onset bactericidal activity with a membrane-targeting mode of action. An alanine scan has revealed that most amino acids are important for the activity.

Outlook

- Generate more derivatives using single point modifications (**LM6**)
- Focus on the insertion of lysine amino acids and secondary structure analysis
- Determine the secondary structure of the peptides by CD
- Explore the other active regions within the peptide further
- Investigate the mode of action in more depth on both GP and GN
- Study resistance development under pressure

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