Designing a new antimycobacterial peptide to tackle *Mycobacterium avium*

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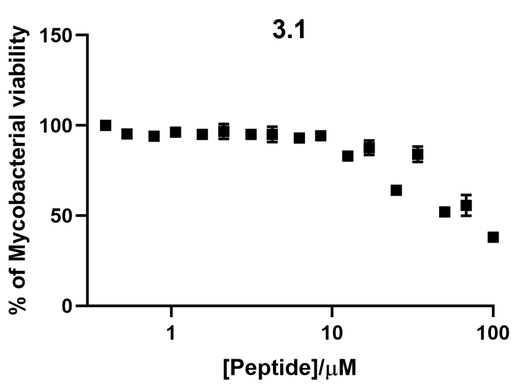
Introduction

Nontuberculous mycobacteria (NTM) infections have been increasing worldwide over the last three decades, with *Mycobacterium avium* complex (MAC) being the most prominent NTM infection around the world. Infecting immunosuppressed individuals and people with chronic respiratory distress, MAC can cause both pulmonary and disseminated infections. The existing treatments for these infections are not only highly prone to fail but also long, costly and toxic due to the cocktail of various antibiotics used. Thus, new therapeutics are required. Antimicrobial peptides (AMP) are a promising alternative approach to these infections not only due to their direct activity by disrupting the microbial cell envelope but also by their immunomodulatory effects. As such, understanding how the activity of these peptides can be optimized is very beneficial. Therefore, the aim of this work was, based on previous work by us[1] and others using AMP against *M. avium*, to understand not only what are the key structural and physiochemical properties determining the antimicrobial effect of a peptide. Taking advantage of *in silico* predictors of AMP's antimicrobial activity and toxicity, three peptides were designed. One was chosen for synthesis and subsequent testing against *M. avium in vitro.*

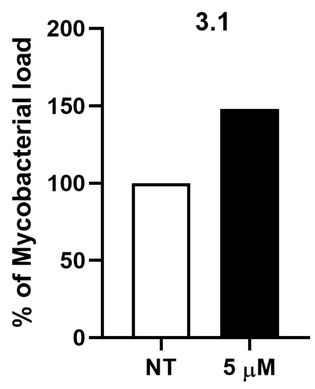
Framework

1. 3.1 – Good antibacterial peptide[2], however

- Ineffective against axenically growing *M. avium* 2447 SmT



- Ineffective against intracellular growing *M. avium* 2447 SmT on bone marrow-derived macrophages



LFcin17-30, peptide with reported efficacy against *M. avium 2447 SmT* [1]

Peptide	Sequence	Charge	Amph	IC_{50} (μM)
3.1	KKLLKWLLKLL-NH_2	4	1.33	76.3
LFcin17-30	FKCRRWQWRMKKLG	6	1.40	14.2 ± 1.5

Why does LFcin17-30 possess such a good activity and 3.1 does not when it has been reported as such a good AMP against both Gram-positive and Gram-negative bacteria?

2. Characteristics of antimycobacterial peptides[3,4]

- Basic
- Amphipathic
- High composition of W, K, R and L
- Preferred amino acids positions

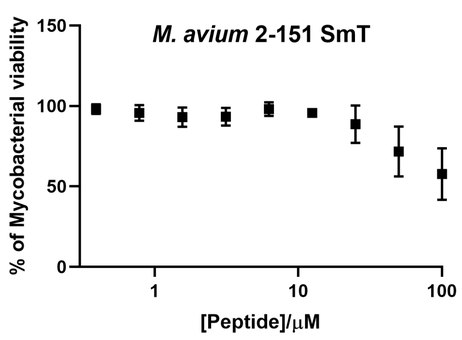
	N-terminal	C-terminal
1	Arginine (R)	Leucine (L)
2	Leucine (L)	Arginine (R)
3	Leucine (L)	Tryptophan (W)
4	Arginine (R)	Leucine (L)
5	Leucine (L)	Leucine (L)

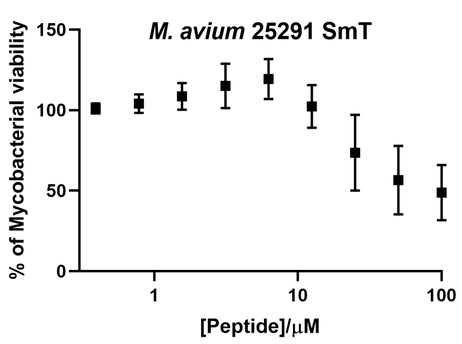
The design of 3 antimycobacterial peptides from LFcin17-30

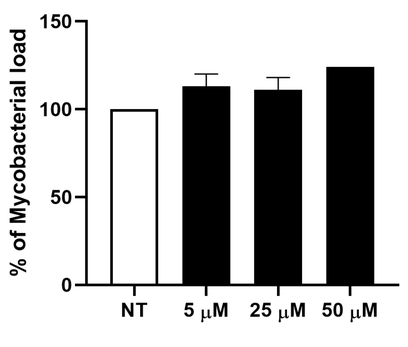
	Sequence	AntiTb Score[a]	ToxinPred Score[b]	Charge	Amph[a]
	FKCRRWQWRMKKLG	1.68	-0.67	6	1.40
GO1	**A**KRW**WA**WRKK**R**L	2.24	-0.91	6	1.53
GOALA1	A**LA**KRWWAWRKKRL	2.40	-0.87	6	1.31
GOALA2	ALAKRWWAWRKK**LL**	2.51	-0.92	5	1.14

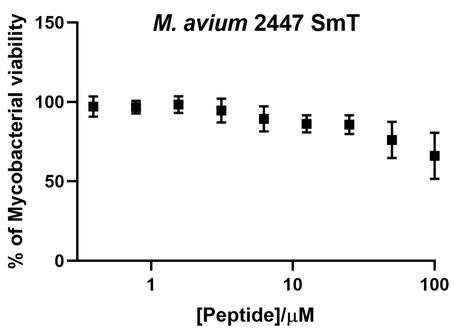
[a] AntiTbPred – Peptide anti-tubercular activity predictor based on a SVM learning model [4]
[b] ToxinPred – Peptide toxicity predictor based on a SVM learning model [5]

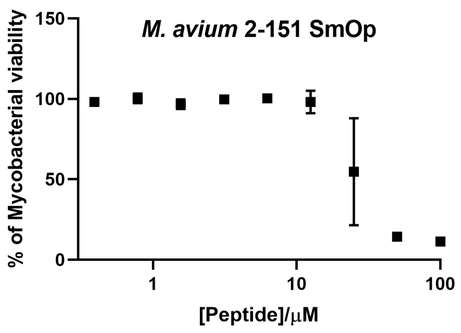
3. GOALA1 activity assessment











- Ineffective against 3 different SmT strains of axenically growing *M. avium*
- Moderately effective against axenically growing *M. avium* 2-151 SmOp
- Ineffective against intracellular growing *M. avium* 2447 SmT

In conclusion

In spite of *in silico* predictors great potential

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GOALA1 failed to have meaningful activity against *M. avium* despite being designed to that end

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***In silico* predictors require extensive negative data in order to be as accurate as possible**

References

1. **Silva T *et al.* (2014) *Antimicrob Agents Chemother*. 58:3461**
2. **Gomes A *et al.* (2019) *Front. Microbiol. 10:1915***
3. **Abedinzadeh M *et al*. (2014) *J Antimicrob Chemother*. 70:1285**
4. **Usmani SS *et al.* (2018) *Front Pharmacol*. 9:954**
5. **Gupta S *et al.* (2015) *Methods Mol Biol*. 1268:143**

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