Whipworm host defence peptides – novel opportunities for parasite control?

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1. Introduction

- Host defence peptides (HDPs) are key components of the invertebrate innate immune system where they provide protection against microbial threat.
- In the context of parasitic helminth infection, worm-derived HDPs may play a critical role in modulating host-parasite-microbiome interactions, particularly in microbe-rich environments such as the gastrointestinal tract.
- Enhanced understanding of the *Trichuris* HDPs may inform drug/vaccine discovery pipelines for pathogen control.

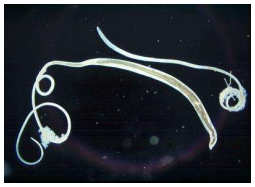

Fig. 1. *Trichuris muris* (mouse whipworm).

2. Aims

To develop a HDP discovery and validation pipeline that integrates (i) an 'omics to characterise HDP profiles in *Trichuris* species, (ii) microbiological analyses to investigate the antimicrobial activity of *Trichuris*-derived HDPs and (iii) develop a cell-based assay to investigate whether these HDPs have immunomodulatory potential.

3. Developing a pipeline to identify HDPs within the *Trichuris* peptidome

The *de novo* dataset contained 5212 predicted peptides. An initial filtering step to remove small (<10 residue) and duplicate sequences resulted in 3047 peptides, which were run through 7 antimicrobial prediction tools. Peptides predicted to be antimicrobial in 5 or more tools (n= 106) were prioritised for further study. The final filtering step involved running the peptides through two additional prediction tools, resulting in 15 candidates to be taken forward for *in vitro* antimicrobial screening (box 5).

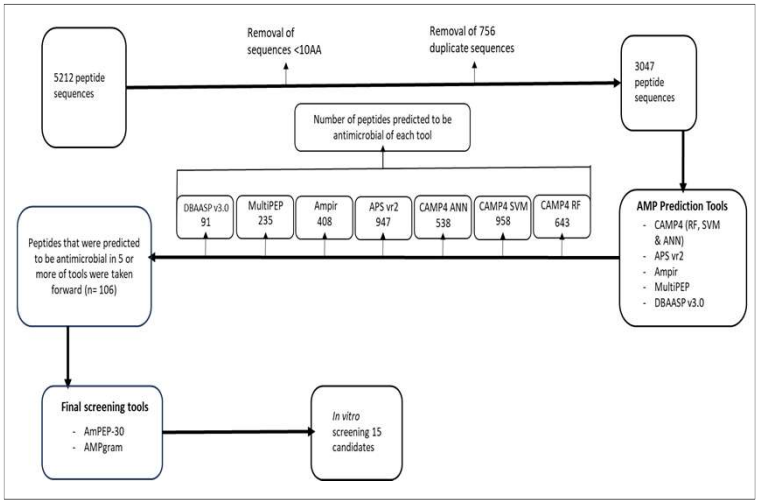

Figure 1. Pipeline to identify HDPs within the *Trichuris* peptidome

4. Identification of peptides with predicted antimicrobial properties within *T. muris* E/S

T. muris E/S was passed through a 10 kDa spin filter and the resulting micro-E/S was analysed by mass spectrometry. 5 peptides (highlighted in green) were identified with high confidence (Table 1.) and were prioritised for further study.

Protein Accession	Peptide	-10lgP	Sample % (N=3)	PTM
Tm-nAMP-LP-7741	Q(-17.03)LPPHGRPFGPVRPPMPG	166.82	100%	Pyro-glu from Q
	S(+79.96)HEADLTSPFAGESH	115.23	67%	Sulfation
	VRPPIRPPYRPLPG	114.57	100%	
	GPVRPPMPG	114.48	100%	
	GPVRPPMP	107.82	33%	
	FGPVRPPMPG	96.32	100%	
	RPPLRPLPGPRPPYRPLPG	94.7	67%	
	Q(-17.03)LPPHGRPFGPVRPPMP	92.96	33%	Pyro-glu from Q
	RPPIRPPYRPLP	91.61	67%	
	Q(-17.03)LPPHGRP	90.28	100%	Pyro-glu from Q
	Q(-17.03)LPPHGRPF	85.43	100%	Pyro-glu from Q
	RPPLRPLPGPRPP	84.28	67%	
	FGPVRPPMP	77.28	100%	
	RPPIRPPYRPLPG	69.87	33%	
	Q(-17.03)LPPHGRPFGPVRPP	63.69	33%	Pyro-glu from Q
Tm-nAMP-LP-7746	HDLHQHHH	127.33	33%	
	DLHQHHH	78.7	33%	
	RPPPPHH	68.14	33%	
Tm-nAMP-LP-7723	GKIWPKQPVPIFT	99.27	67%	
	WPKQPVPIFT	94.77	33%	
	APPDNQTGSKRA	82.94	33%	
	Q(-17.03)PVPIFTYKRTF	67.94	33%	Pyro-glu from Q
Tm-nAMP-LP-7745	LPQGGVFLPMIQR	99.54	33%	
	SLPQGGVFLPMIQR	78.33	67%	
	GVFLPMIQR	70.22	33%	
Tm-Nem-2	W.GVIQGEQDGTSNFAFTDAKK.M	145.38	33%	
Tm-Nem-1	G.GVIQGEQDGTSNFAFTDAKK.M	145.38	33%	
Tm-nAMP-LP-1545.2	ICGKPYYKPILPTKDHVGNR	82.43	33%	
	ILPTKDHVGNR	77.96	100%	
	KPILPTKDHVGNR	61.22	33%	
Tm-nAMP-LP-1545.1	HPPPPPIHH	80.19	100%	
	HPPPPPIH	77.86	33%	
	GPGHHPPPPVH	63.71	33%	

Table 1. Mass spectrometry data suggesting the existence of parasite-derived HDPs within *T. muris* micro-E/S. Peptides highlighted in green were identified with high confidence and were prioritised for further study.

5. Investigating the antimicrobial properties of *Trichuris* HDPs.

The 5 peptides identified in Table 2 were chemically synthesised and a microbroth dilution assay was used to determine the minimum inhibitory concentration of each peptide against representative members of the gut microbiota. None of the proteins identified in box 4 demonstrated antibacterial activity (Table 2). Those identified in box 3 are yet to be tested.

Peptide	Efficacy (Gram-)	Efficacy (Gram+)
7741	X	X
7723	X	X
7745	X	X
1545.1	X	X
1545.2	X	X

Table 2. Synthetic micro-E/S derived HDPs do not exhibit antibacterial properties.

6. Investigating the immunomodulatory properties of *Trichuris* HDPs.

Work is ongoing to develop a cell-based assay to determine whether any of the synthetic micro-E/S peptides (or those identified in process outlined in box 3) have immunomodulatory properties.

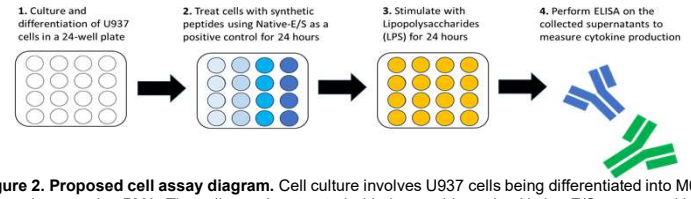

Figure 2. Proposed cell assay diagram. Cell culture involves U937 cells being differentiated into M0 macrophages using PMA. The cells are then treated with the peptides using Native-E/S as our positive control. The cells are then stimulated with LPS for 24 hours before the supernatant is collected and ran through an ELISA to measure cytokine production.