Hide and Caecum: Looking for antimicrobials in the Equine tapeworm, *Anoplocephala perfoliata*

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Introduction

Gastrointestinal (GI) helminths have been observed to create significant changes to the GI microbiome with which they share their environment[1]. Helminths, largely nematodes, have also been shown to create a plethora of antimicrobial peptides (AMPs) secreted though extracellular vesicles (EVs) and secretory products (ESP) which has been hypothesised as a mechanism for host-microbiome changes[2]. *Anoplocephala perfoliata,* an equine tapeworm, has often been neglected amongst molecular research. However, the recent generation of a transcriptome and secretory proteomes (ESP & EVs) provides a foundation for greater understanding for its host-parasite relationships[3]. Thus, this project aims to investigate the potential antimicrobial nature of *A. perfoliata* EVs.

Fig 1. *A. perfoliata*.

Bioinformatic Investigation

Bioinformatic comparison of *A. perfoliata*, *Calicophoron daubneyi*, and *Fasciola hepatica* transcriptomes & EVs was carried out to identify potential AMPs using multiple pipelines (See Tables #, #). In total, 34, 130, and 13 unique IDs were identified in *A. perfoliata*, *C. daubneyi*, and *F. hepatica*, respectively (**Table #.**). Additionally, 6, 8, and 4 of these retrieved IDs were also identified in their EVs, respectively (**Table #.**)

Table #. Potential AMPs identified in the transcripts of three platyhelminths.

Spp.	Description	Method	No.
A. perfoliata	Saposin like	Homology	3
	α-Hairpinin	Cysmotif	4
		Cysmotif	1
	Defensins	Cysmotif	2
		Cysmotif	1
	CYSRICH	Cysmotif	15
C. daubneyi	SAPLIPs	Homology	43
	PGRPs	Homology	11
	α-Hairpinin	Cysmotif	10
		Cysmotif	8
	Defensins	Cysmotif	1
		Cysmotif	3
	THI019	Cysmotif	3
	CYSRICH	Cysmotif	44
F. hepatica	Saposin like	Homology	3
	PGRPs	Homology	3
	α-Hairpinin	Cysmotif	1
	Defensins	Cysmotif	1
	THI019	Cysmotif	1
	CYSRICH	Cysmotif	1

Table #. Potential AMPs identified in the EVs of three platyhelminths. [W] Whole, [S] Surface.

Spp.	Description	Method	No.
A. perfoliata	α-Hairpinin	Cysmotif	1[W]
	Ubiquitin	CAMP R3	2[W]
	ACoA-binding	CAMP R3	2[W, S]
	Mastin	CAMP R3	1[W]
	Thioredoxin-like fold	Ampir	1[W]
	Uncharacterised	Ampir	2[W]
C. daubneyi	SAPLIPs	Homology	3[W]
	Ubiquitin	CAMP R3	3[W]
	Histone	CAMP R3	2[W]
	Lysosomal aspartic protease-like	Ampir	1[W]
	Uncharacterised	Ampir	1[W]
F. hepatica	Saposin like	Homology	1[W & S]
	PGRPs	Homology, CAMP R3	1[S]
	Ubiquitin	CAMP R3	2[W, S]
	Mastin	CAMP R3	1[S]

Antimicrobial Assays

Optical density assays were performed in 96 microwell plates using 10 µg of *A. perfoliata* EVs or ESP with a gram negative (*Escherichia coli*) or gram positive (*Bacillus megatirium*) bacteria. NTA analysis demonstrated 10 µg equates to: EV1 = $4.7E^{+10}$, EV2 = $6.1E^{+10}$; EV3 =$6.2E^{+10}$. No antimicrobial effect from EVs was observed against either strain (**Figure #.**)

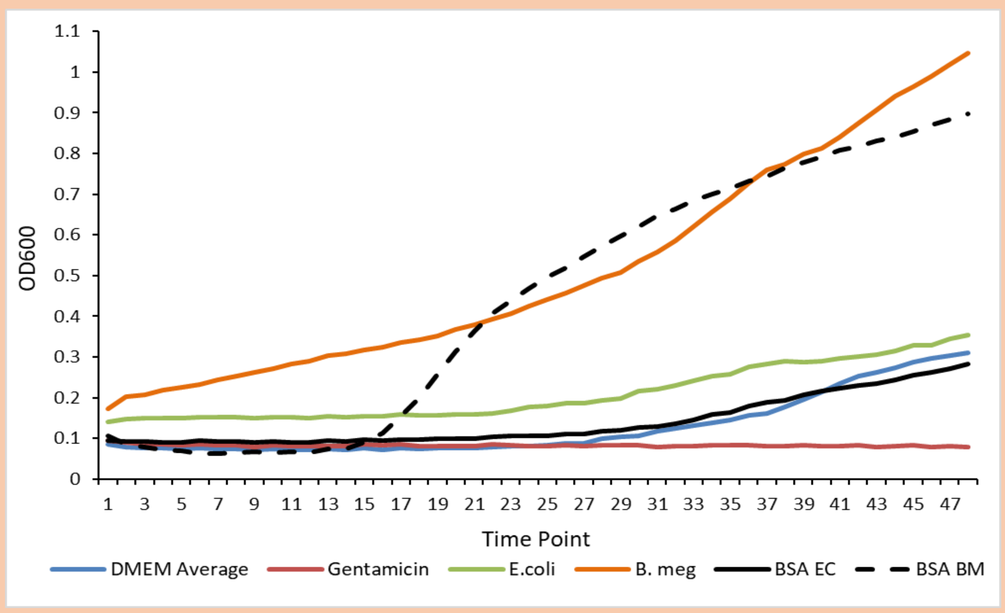

Fig 4. OD of *A. perfoliata* EVs and ESP against *E.coli and B*. megatirium. Negative control, bovine serum albumin (BSA EC: *E. coli* and BSA BM: *B. megaterium*). Positive control: gentamicin.

Conclusions

The current data suggests that *A. perfoliata* have the ability to produce a range of different potential AMPs which can be localised within their EVs. However, little antimicrobial activity demonstrated against both a Gram-negative and Gram-positive species may suggest ApEVs, and their potential AMP play another role such as immune modulation. We continue to explore the role *A. perfoliata* EVs may have in in altering the microbial environment within the horse host caecum and wider GI tract.

Somatic and >10kDa EV-free ESP AMPs

A. perfoliata fractions (Somatic, & EV, EV-depleted ESP) characterised via 12.5% 1D SDS-PAGE gels and LC-MSMS and MASCOT MS/MS Ion Search against the *A. perfoliata* transcriptome[3]. Somatic and ESP fractions were investigated for potential AMPs as previously done with EVs (Table #.).

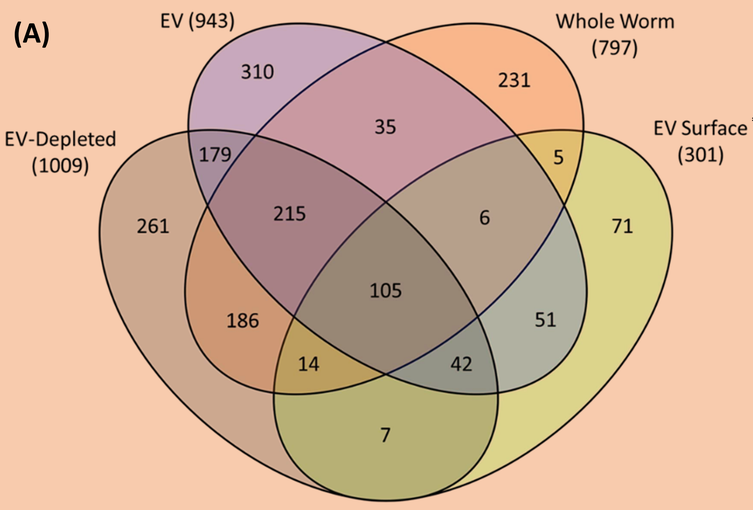

Figure #. Comparison of *A. perfoliata* somatic, EV whole , EV surface, & ESP proteome data. * EV surface IDs were retrieved from Wititkornkul et al. (2021) [3]. (B) TEM image of *A. perfoliata* EVs.

Table #. Potential AMPs identified in the Somatic and > 10kDa EV-free ESP fractions of *A. perfoliata*.

Spp.	Description	Method	No.
Somatic	Ubiquitin	CAMP R3	2
	Thioredoxin-like fold	ampir	1
	1,5-anhydro-D-fructose reductase	ampir	1
EV- free ESP	α-Hairpinin	Cysmotif	1
	Mastin	CAMP R3	3
	Ubiquitin	CAMP R3	2
	ACoA-binding	CAMP R3	1
	Ca-binding	ampir	1
	Thioredoxin-like fold	ampir	3
	Hypothetical transcript	ampir	2
	carbonyl reductase 1	ampir	1
	Dynein light chain	ampir	1
	Cell division control protein	ampir	1
	Cytidine deaminase	ampir	1
	1,5-anhydro-D-fructose reductase	ampir	1
	Aldo keto reductase	ampir	1
	immunogenic protein	ampir	1

Peptidomics

Steps have been taken to identify the *A. perfoliata* peptidome. *A. perfoliata* fractions (EV, ESP, & Somatic) have been prepared via the acidic methanol method[4]. Initial summary of EV peptidome results are shown below…

- 188 unique transcript IDs – 224 potential protein frames
- 15 expressed conserved protein
- 5 unnamed protein products
- 6 hypothetical protein/transcripts
- 1 Uncharacterised protein
- 81 No BLAST
- Protein Examples - Dynein light chain, P29, calpain A, protein AHNAK2, Glyceraldehyde-3-phosphate dehydrogenase, Heat shock 70 kDa protein

Peptides are currently being further characterised for function and for antimicrobial potential.

Future Work

- Further explore *A. perfoliata* peptidome – Somatic & ESP, AMP analysis.
- Expand antimicrobial assays to include physiologically relevant gut-microbes, such as *Prevotella spp*. *Clostridium spp*., and *Selenomonas spp*.
- Gas fermentation model utilising equine caecal material with incubation of *A. perfoliata* EVs and EV-free ESP.

References

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