Ascaris suum Pseudocoelomic Fluid: A Peptide-rich Biofluid that Modulates Nematode Motility

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Bioactive peptides, including Neuropeptide-like Proteins (NLPs), FMRFamide-like Peptides (FLPs) and Insulin-like Peptides (ILPs), are known to influence key nematode neuromuscular functions such as locomotion, feeding and egg laying. The wired component of the nematode neuropeptide signalling system is well characterised, however non-synaptic routes of communication are less well known. Nematode pseudocoelomic fluid (PCF) may serve as an alternative, non-synaptic, route of peptide transmission. Unfortunately, the size-related intractability of most nematodes has prevented the characterisation of the PCF peptidome, however the large size of the pig parasite *Ascaris suum* offers an opportunity to achieve this in a nematode pathogen. A recent LC-MS/MS study detected 76 peptides (FLPs, NLPs and AMPs) in *Ascaris* PCF (As-PCF) and demonstrated that As-PCF is bioactive on nematode muscle. In this study we have employed *in silico* bioinformatics (HMM/BLASTp), Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS), Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) and nematode bioassays to expand on these analyses by examining the ILP component of As-PCF and the bioactivity of As-PCF on nematode behaviour. The data demonstrate that: (i) 409 Insulin-like Peptide (ILP) sequelogs are present in 109 nematode species (262 novel ILPs); (ii) 9 ILP sequelogs are present in *A. suum*; (iii) As-PCF contains 90 peptides including 7 NLPs, 4 FLPs, 76 AMPs and 3 ILPs (ILP-1, -18 and -31); (iv) As-PCF significantly reduces *Caenorhabditis elegans* motility. These data have expanded the peptide library used to mine As-PCF LC-MS/MS data, documented the presence of ILPs in As-PCF, and move towards the identification of the bioactive peptide components of As-PCF. Further characterisation of the As-PCF peptidome will enhance our understanding of nematode biology, including non-synaptic peptide transmission, and may inform drug development strategies for parasite control.