Isolation and characterisation of genes expressed during the early stages of insect infection by *Steinernema carpocapsae*

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Introduction

Steinernema carpocapsae is a soil dwelling entomopathogenic nematode, commonly used as a biological control agent. The infective stages, known as the dauer juveniles (DJs) carry in their intestine a symbiotic bacterium – *Xenorhabdus nematophila*. In general entomopathogenic nematodes require their symbiont to kill their insect host, but axenised *S. carpocapsae* are able to infect and kill unaided. Hence it is believe that S. carpocapsae possesses its own genes encoding proteins toxic to the insect.
After the nematode infects the host it goes through a "recovery" stage, it is at this stage that genes encoding toxins are likely to be most abundantly expressed.

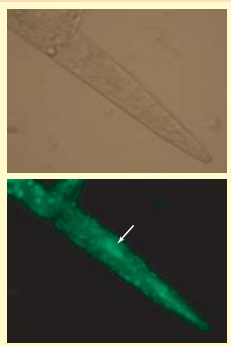
The aims of this project are:
1. To identify strains of *S. carpocapsae* that are highly virulent against insect hosts
2. To identify tissues in the recovering nematode that are transcriptionally active
3. To identify novel genes encoding proteins toxic to the insect host

Recovering dauer juveniles of S. carpocapsae

Life cycle of S. carpocapsae

SYTO 12 staining

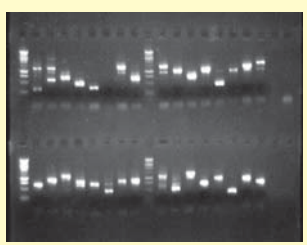
- SYTO 12 is a fluorescent dye that emits light when bound to RNA.
- Binding of SYTO 12 to a particular cell or tissue is indicative of high levels of transcription within that tissue.
- Binding of SYTO 12 is observed using fluorescence microscopy.
- We examined binding of SYTO 12 to recovering juveniles of *S. carpocapsae*
- The stain bound to the pharyngeal glands and genital primordia, indicating that these tissues are transcriptionally active.
- Activity in the gland cells suggests that these cells are actively synthesising large quantities of secretory proteins that may be insect toxins at this stage.
- Presence of RNA was highest 4 hours after recovery, Nematodes at this stage are most likely to be abundantly expressing genes toxic to the insect host and were therefore selected for further analysis.



SYTO 12 staining of S. carpocapsae juvenile 4h after recovery. Light image (upper panel) shows nematode anterior tip. Lower panel shows fluorescence from SYTO 12 in the gland cells (arrow).

Analysis of a cDNA library of recovering *S. carpocapsae* dauer juveniles

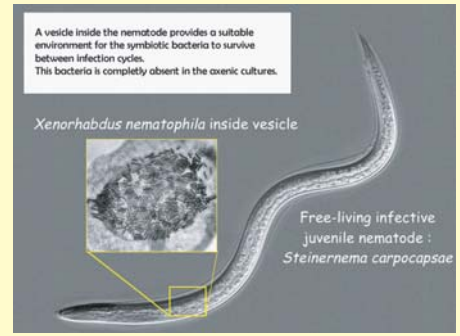
- A cDNA library was made from mRNA extracted from nematode strain Breton 4 hours after induction of recovery using a Creator SMART cDNA Library Construction kit (BD Biosciences).
- Over $1x10^6$ primary recombinants were present after cloning, suggesting the library is representative of genes expressed in *S. carpocapsae* dauers.
- Analysis of insert size (see figure right) shows a good range of insert sizes are present in the library and that the average insert size is well in excess of 500bp.
- 15,360 colonies were picked from this library into 384 well plates using a Q-bot robot (Genetix)
- ESTs are being sequenced from this library – analysis of the first 1000 clones showed that the library is not yet redundant. A further 4000 clones are therefore being sequenced.



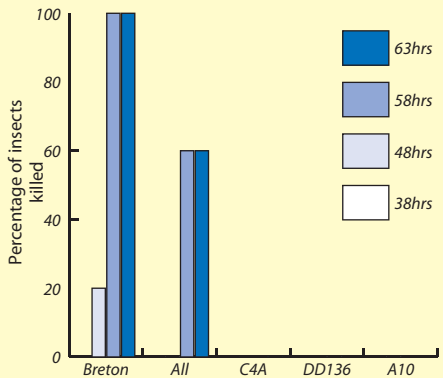
Results of PCR reaction examining insert size in S. carpocapsae *cDNA library. Markers are Promega 1Kb ladder.*

S. carpocapsae can kill the insect host in the absence of symbiotic bacteria

- Previous studies have shown that *S. carpocapsae* can infect and kill its insect host in the absence of its symbiotic bacteria *X. nematophila* (Simoes et al., 2000: J. Invert. Pathol. 75, 47-54)
- By contrast, axenic dauer juveniles of *Heterorhabditis bacteriophora* are unable to kill *Galleria mellonella* (waxmoth larvae); they require the symbiotic bacteria *Photorhabdus luminescens*. This nematode is dependent on its symbiont to complete its life cycle
- The fact that *S. carpocapsae* is pathogenic to insects in the absence of symbiotic bacteria strongly suggests that it possesses genes that encode insecticidal proteins. These genes are usually encoded in the genome of the bacterial symbiont in other EPN pathosystems.



Identification of virulent strains of *S. carpocapsae*



Virulence of S. carpocapsae *strains against* Galleria mellonella

- Five strains of *S. carpocapsae* were collected and axenic cultures of each strain were established.

Strain	Origin
All	USA
Breton	France
C4A	China
DD136	USA
A10	USA

- Toxicity tests were conducted on *Galleria mellonella* to identify the most virulent axenic strain of *S. carpocapsae*
- Dauer juveniles were injected into *G. mellonella* at doses of 1,5 and 10 DJs/*Galleria*
- The strain Breton was found to be the most virulent killing 100% of exposed insects at a dose of 1DJ/*Galleria* within 53 hours (see bar graph left).
- This kill rate and time is comparable to that of the intact nematode - bacterial complex.

Future work

- Complete sequencing of 5000 ESTs from the *S. carpocapsae* cDNA library.
- Bioinformatic analysis of ESTs – this will include contig analysis followed by BLAST searching.
- Functional analysis of selected genes using techniques including RNAi and heterologous expression followed by *in vitro* testing.
- Priorities for functional analysis will include putative genes that enable DJs to kill insects unaided and other developmentally regulated genes involved in the transition from dauer larvae to the parasitic stage in *S. carpocapsae*.

Acknowledgements

Funding for this work was provided by Science Foundation Ireland. COST Action 850 also provided funds for a short term visit by Zoe Mulroy - Hehir to SCRI. SCRI receives funds from the Scottish Executive Environment and Rural Affairs Department. The technical support of Claire Booth (SCRI) and Ica Dix (NUI Maynooth) is gratefully acknowledged.