

Proteins expressed by *Steinernema carpocapsae* in infection process

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Introduction

- *S. carpocapsae* is able to invade, to escape and to kill insects in a few hours post contact.
- Mainly the IJ invades haemocoelium by the digestive tract, thus passing natural barriers like peritrophic membrane and gut epithelium.
- Despite IJ are recognized and encapsulated by some insects, most of them are able to overcome cellular and humoral host defenses, suggesting an efficient control of host defenses.

Introduction

- Insect death occurs within 2 days of parasitism, thus evidencing the expression of toxic factors.
- In this work we continued an investigation on the identification of proteins involved on pathogenesis of *S. carpocapsae*.

Experimental procedures

Nematodes

Induction

Secreted

Products (S/P)

Disinfection and remove de cuticle of Dj (resistant stage)

Induction in SF900 (insect cells culture medium)

6h

18h

Transfer to PBS to collect the S/P

Concentrated with centricon® and precipitated in TCA/DTT

Homogenate
total organisms

Disinfection and remove de cuticle of Dj

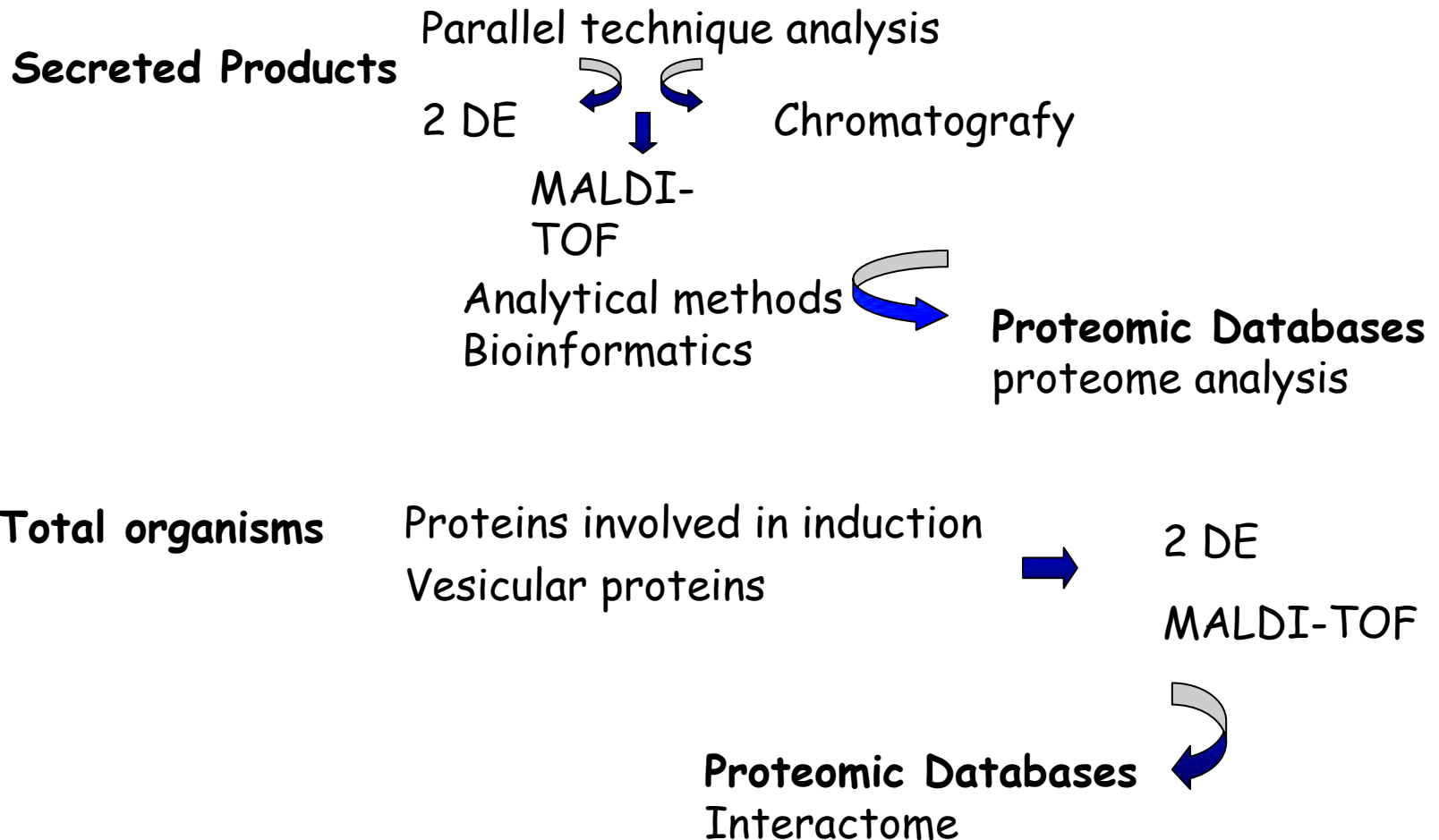
0h

6h

Powdered in a pestle and mortar under liquid nitrogen

Homegenization with extraction buffer (Ureia9,8M/ CHAPS 4%)

Experimental procedures



Experimental procedures

Integration of four important tools:

➔ Analytical protein separation technologies

Isoelectric focusing, IPG gels
Second Dimensional
Electrophoresis in SDS-gel
Cromatografy

➔ Mass spectrometry (MS)

MALDI-TOF analysis

➔ Databases (proteins)

Mascot Search Results

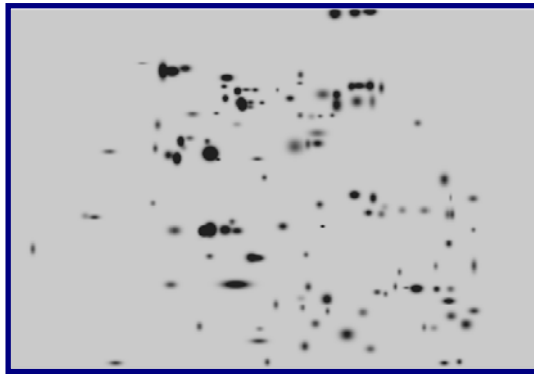
Bioinformatics

2DE whole Organism

Differential proteome analysis

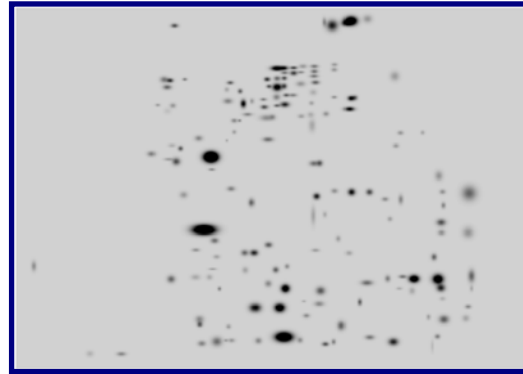
Non Induced

123 spots

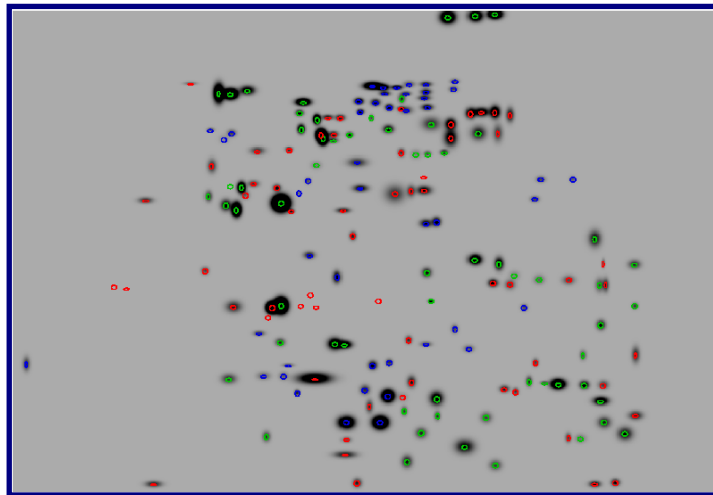


Induced

159 spots



Match 40%
46 new spots
53 spots lost



Red spots gifts only in DJ

Blue spots gifts only in IJ

Green the common ones

Image generated for PDQuest software from the image standard of the gels 2-D gotten for the two physiological situations.

2DE Secreted Products

6h → 56 spots

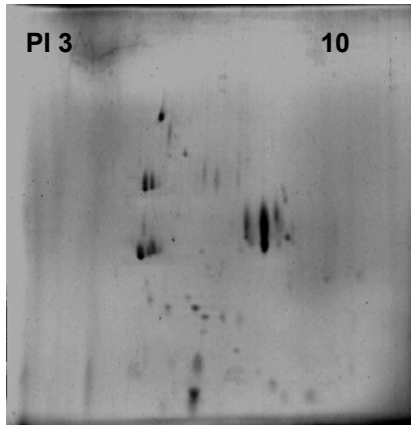
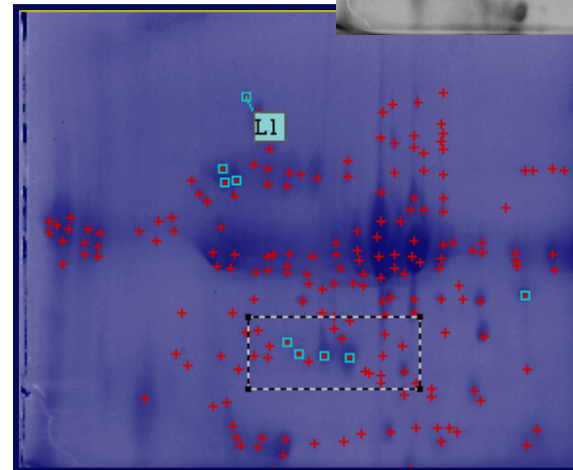
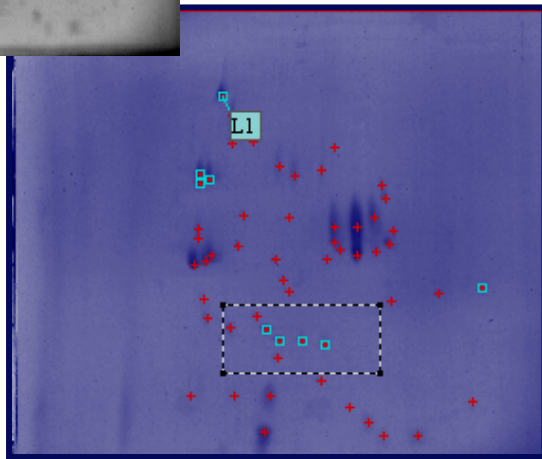
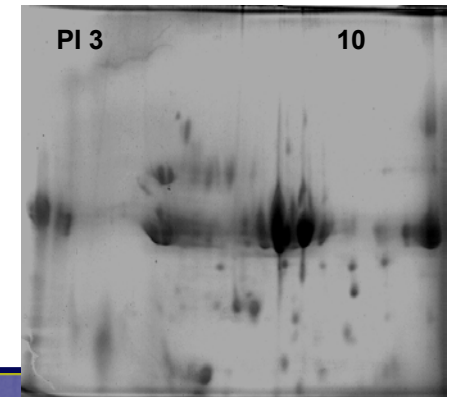


Image Software Analysis
Protein detection

- 13 spots disappear
- 136 new spots
- 33% matches

18h → 202 spots

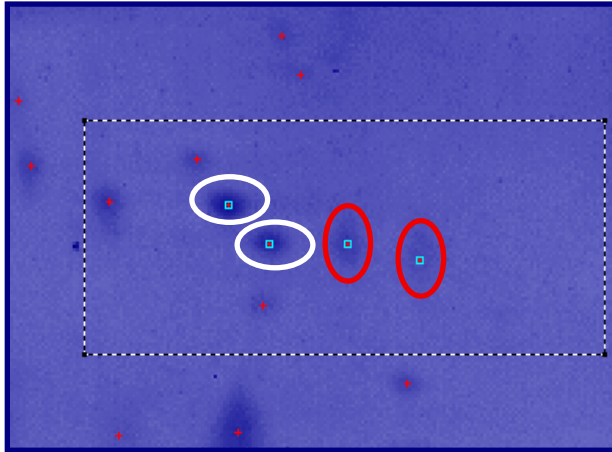


For imagemaster software image analysis (Amersham)

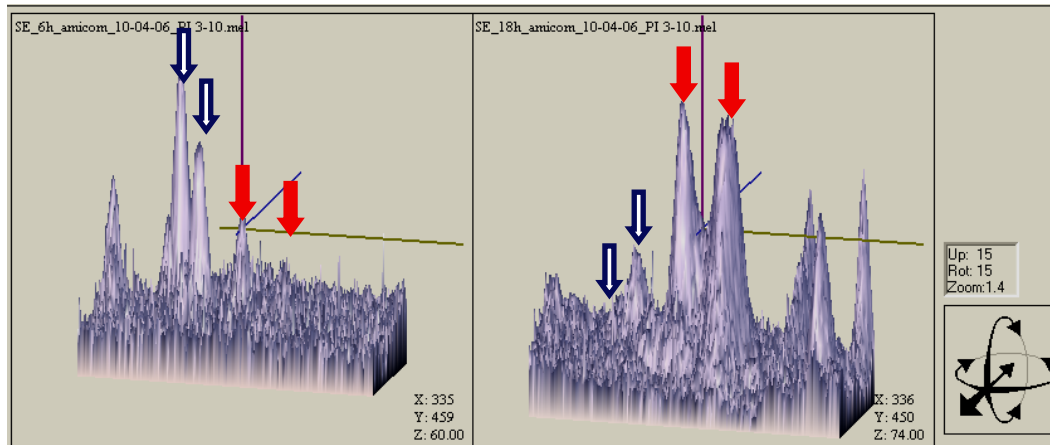
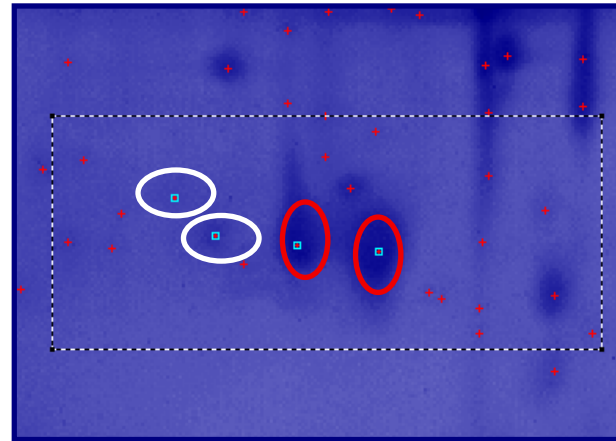
2DE Secreted Products

Protein quantification
Differential proteome analysis

SP 6h

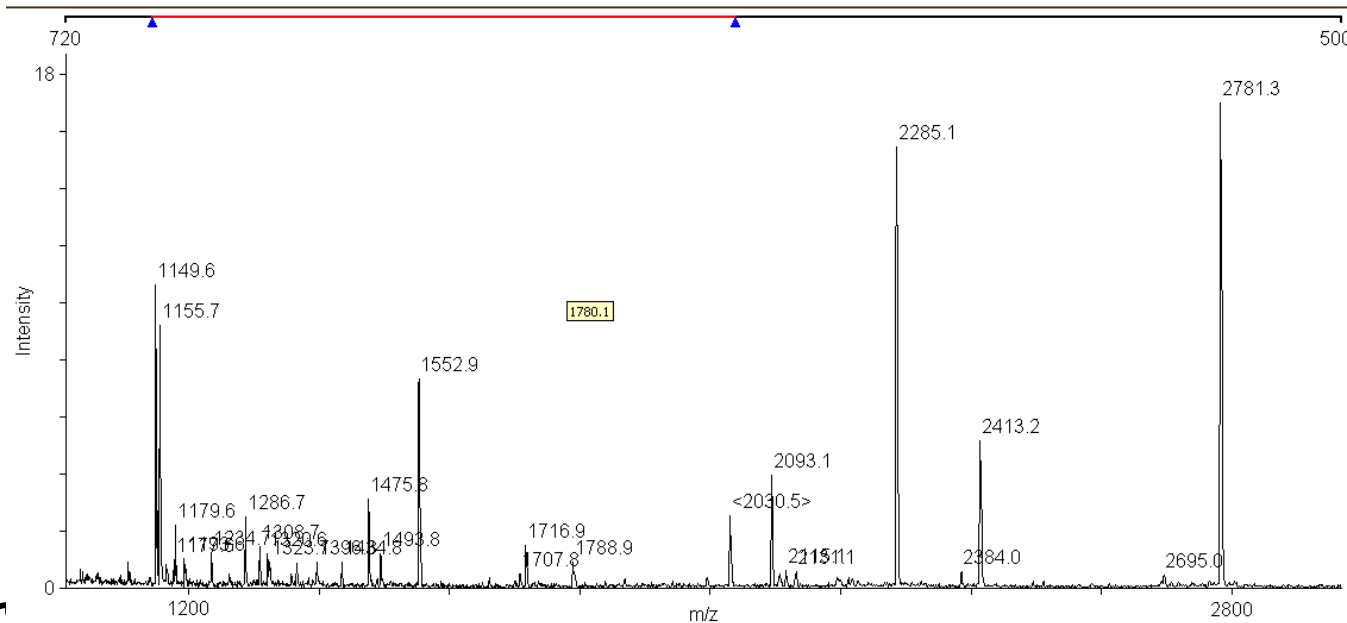


SP 18h



For imagemaster software image analysis (Amersham)

MALDI-TOF analysis

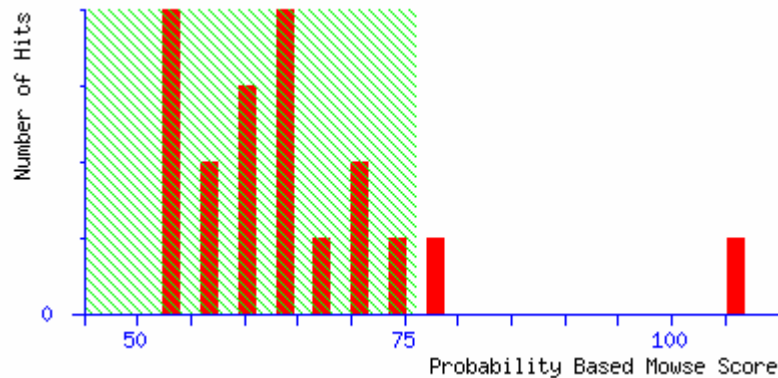


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Mascot search
match results



IDN_GENBANK

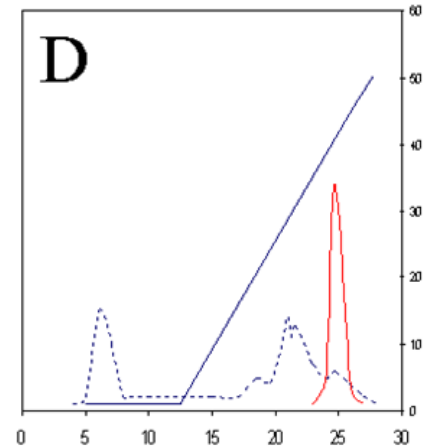
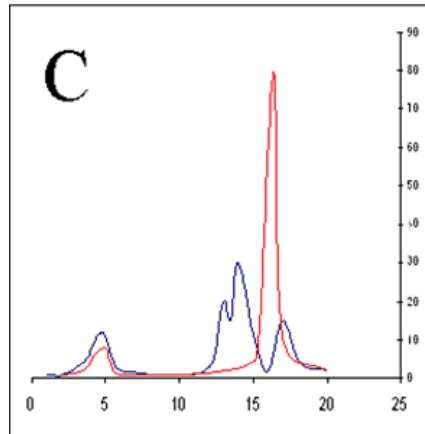
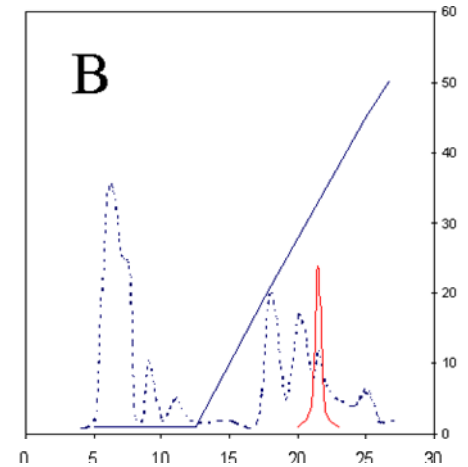
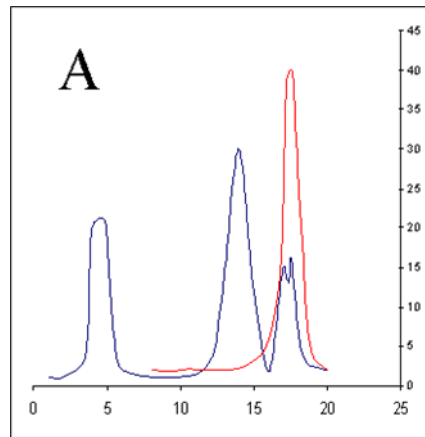
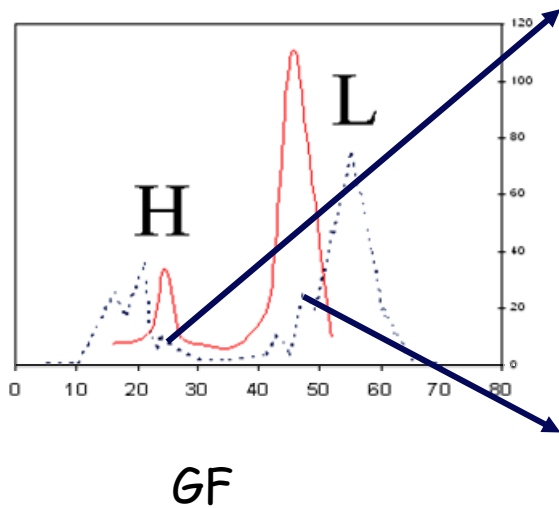


Proteins matched in SP

- Proteases:
 - Quimotripsin
 - Elastases
- Proteins involved in the regulation of apoptosis
- Proteins involved in anti-oxidant processes
- Elongation factors
- Antifreeze glycopeptide
- ... ~ 100 spots analyzed

SP separation / purification

Ser-proteases - (Chromatography)

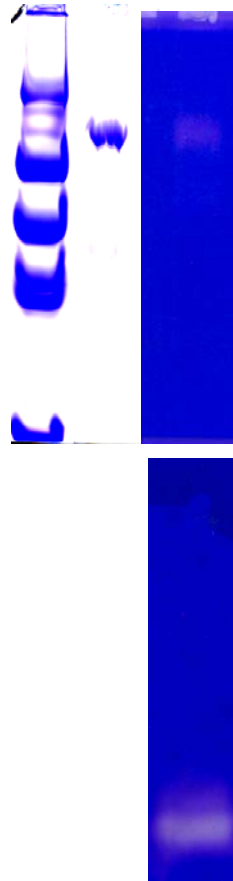


Affinity

Mono Q

SP separation / purification

SDS Zimogram



Pick H

Quimiotripsin-
protease

~ 60kDa

Pick L

Elastase- protease

~ 30 kDa

Discussion

- Proteins detected in the homogenate of **non induced nematodes** must be related with control of arrested stage, namely nematode-bacteria symbiosis.
- Proteins detected in homogenates of **induced IJ** must be correlated with nematode grow and infection.
- Proteins detected on **secreted products**, part of them, must interact with insect (virulence factors ?).