Molecular and Genetic Studies of the Basis of Virulence/Avirulence in *Meloidogyne chitwoodi*

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Abstract

Within the EU project DREAM there is a programme of work investigating the molecular basis of virulence and avirulence in *Meloidogyne chitwoodi* and which involves partners from the Netherlands, France and Scotland. In Wageningen AFLPs are being used to examine genetic diversity between populations of *M. chitwoodi*. Partners in Wageningen and INRA are using *M. javanica* and *M. incognita* as model systems to isolate genes producing secreted proteins that could be implicated in the host pathogen interaction. They will search for homologous sequences in *M. chitwoodi*. At Rennes two-dimensional electrophoresis (2DE) studies are being conducted. The PCR based suppressive subtractive hybridisation (SSH), has been used at PRI and SCRI to compare avirulent and virulent nematodes and infected plant material (resistant and susceptible) at different times after infection.

Keywords: *Meloidogyne chitwoodi*; potato; AFLP; cDNA; suppressive subtractive hybridisation (SSH); two-dimensional electrophoresis (2DE)

INTRODUCTION

The general objective of the DREAM project is to develop control strategies for utilising durable resistance to soil-borne pests. *Meloidogyne chitwoodi* and *M. fallax* were chosen as a model systems. They are quarantine pests and present an increasing threat to European agriculture. They are able to multiply on many crop plants and show genetic intraspecific differentiation including differences in virulence

This paper describes how part of the project aims to use genetic and molecular techniques to develop new tools for a durable resistance management and is targeted at examining differences between virulent and avirulent nematodes. Genetic studies aim to provide information on the inheritance and stability of resistance and virulence. Molecular techniques and strategies are being developed for the identification of (a)virulence genes in *M. chitwoodi* and *M. fallax* that will enable molecular markers to be developed to characterise these species. Populations can then be characterised allowing informed decisions to be made with regard to cropping strategies and the deployment of resistant cultivars to control the nematodes. In addition the work will aim to identify nematode genes and proteins implicated in the host/pathogen interaction. Some of these genes will be functionally characterised to provide a better understanding of the host/pathogen interaction and to open up possibilities for the development of novel control strategies.

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EXPERIMENTAL APPROACHES AND RESULTS

Genetic studies

Production of progeny from virulent × avirulent genotypes of *M. chitwoodi*

At PRI hybrid populations of nematodes are being made using virulent and avirulent isolates of *M. chitwoodi* as parents. Parents are being characterised using molecular and cytological techniques. The progeny will be biologically tested for virulence and used for inheritance and cytological studies.

Preliminary experiments have shown that controlled *in vitro* crossing of (virulent and avirulent) *M. chitwoodi* lines with equal chromosome number is possible. However as not all offspring will be hybrids (unfertilised eggs develop by parthenogenesis in this species) a test with proper parental markers is needed.

Identification of markers for genetic analysis

AFLPs are being used to fingerprint populations in order to identify differential markers to verify hybridisations. They will also be examined for their association with virulence.

At PRI 21 isolates of *M. chitwoodi* and 9 isolates of *M. fallax*, as well as 3 isolates of each of *M. incognita* and *M. javanica* have been submitted to AFLP analysis. The AFLP banding patterns of the isolates of *M. chitwoodi* and *M. fallax* were variable. Most bands seem to be present in all isolates but differed in banding intensity. Although species specific and isolate specific fragments were obtained so far none of these markers have been shown to be related to virulence. In contrast to the results obtained with *M. chitwoodi* and *M. fallax*, the species *M. incognita* and *M. javanica* could clearly be differentiated by species-specific polymorphic AFLP bands. Future work will focus on the acquisition of AFLP markers related to virulence.

Gene isolation

Three related approaches will be used to identify candidate genes from cDNA:

(1) Identification of differentially expressed genes during infection using cDNA AFLP

In Antibes 2 pairs of near-isogenic *M. incognita* lines have been selected in the laboratory, for their avirulence/virulence against the tomato *Mi* resistance gene. These lines have been compared. 256 primer combinations have been used and have generated about 85 000 AFLP fragments, among which 19 fragments were confirmed as differential. The differential expression of 3 genes from these has been confirmed by RT-PCR. All three showed no homology to known sequences, and presumably are pioneer genes. Currently the full-length cDNAs are being cloned using RACE and Inverse PCR strategies.

At WAU the cDNA-AFLP method is also being used and gene expression profiles of various *M. incognita* stages differing in oesophageal gland activity are being compared. Genes specifically expressed in either the pre-parasitic or the parasitic stages may represent candidate parasitism genes and are being further characterised. The DNA sequences of isolated fragments are being compared with the *M. incognita* EST database using the computer program GenEST (Qin *et al*. 2001). This method has been successful and validated those genes from the oesophageal glands.

(2) Suppressive subtractive hybridisation

The PCR based suppressive subtractive hybridisation (SSH) is another technique being deployed. This approach enables the production of enriched cDNA libraries containing sequences from either virulent or avirulent nematodes using pre-invasive juveniles as well as infected resistant and susceptible plant material at different time points. When using infected plant material, plant genes expressed in the compatible and incompatible interaction are also being isolated.

At PRI SSH has been used to compare two *M. chitwoodi* isolates differing in virulence on accessions of *Solanum bulbocastanum*. Approximately 400 sequences have been obtained with some showing no significant BLAST hit to known proteins or DNA sequences and are possibly unique to the virulent isolate. Other sequences showed (low) homology to nematode EST clones whist others had high homology to plant genes (mostly potato and tomato).

At SCRI roots of *S. bulbocastanum* were infested with two isolates of *M. chitwoodi* differing in virulence. The infection process was monitored by histological examination of roots allowing time points to be identified. cDNA libraries enriched for transcripts from either compatible or incompatible interactions, at three days and seven days post infection were obtained. Both plant and nematode genes were identified.

Genes associated with oxidative processes commonly found during a hypersensitive response were identi-

fied from the avirulent/incompatible libraries whilst genes associated with cell growth and signalling were identified only from the virulent/compatible libraries. Genes associated with sugar metabolism were identified with a higher incidence of such genes being seen in the avirulent interaction. Previously unknown nematode genes were also identified. These genes will now be characterised to determine their role and whether they are differentially expressed in each isolate.

(3) Production of cDNA libraries

The construction of a cDNA library from *M. chitwoodi* juveniles is currently being made and clones from it will be sequenced at random to obtain ~1000 ESTs. Secretory proteins from *M. chitwoodi* will be identified and investigated. This library will also be used to obtain full-length sequences corresponding to fragments isolated using SSH and cDNA-AFLP.

Differential protein analysis using 2 dimensional electrophoresis

An alternative approach for gene isolation is by a comparative study using 2D electrophoresis. Protein analysis of virulent and avirulent nematodes is being conducted and differentially expressed proteins micro- sequenced.

2DGE techniques have been optimised in order to obtain profile production of proteins in a wide pH range (3–6, 5–8, 7–10) and on a larger gel (20 × 20 cm). Proteins have been extracted from mature females and from juveniles. From females approximately 2000 proteins have been identified and only 1123 from juveniles. Work has focused on the differential proteins from juveniles. Some of them seem to be characteristic of the virulence or avirulence of each line. After confirming the value of the protein candidates, the next step will be identification by mass-spectrometry.

Functional analysis

Sequence homology searches are being undertaken to find the presence of signal peptide sequence helping to identify secreted proteins. Expression patterns will be examined and *in situ* hybridisation is being used to establish where expression is located. Verification of the presence of candidate genes in other virulent and avirulent populations will be evaluated to test their utility as markers. The effect on fitness of pathogenicity/candidate genes will be investigated by making knock outs using dsRNA.

References

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