Functional and genetic characterisation of a family of chorismate mutases from the potato cyst nematode *Globodera pallida*.

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Introduction: PCN and chorismate mutase

The potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* cause extensive damage to crops in the UK (Figure 1)

The PCN problem has been exacerbated by the spread of *G. pallida*, which is more difficult to control using natural resistance or nematicides

PCN induces the formation of a syncytium (Figure 2) in the roots of the plants it infests and depends on the syntcytium for all the nutrients it requires to develop to the adult stage

Syncytium formation requires extensive reprogramming of plant gene expression

The developing syncytium also needs to remain undetected by the plant in order to avoid activation of plant defence responses

Understanding the molecular processes used by nematodes to induce and protect their syncytia offers the possibility of new control strategies based on disruption of these processes. Consequently, we are studying the function of secreted proteins that may play a role in these processes.

Figure 1(above). Effect sof PCN on susceptible (upper) and naturally resistant (lower) plots of potatoes

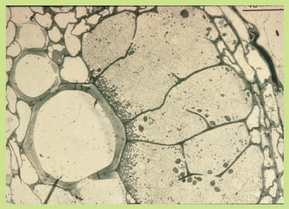

Figure 2 (right). Cross sectiion of a syncytium in the roots of potato

Figure 3. In situ hybridisation reaction showing that expression of the G. pallida chorismate mutase is restricted to the subventral gland cells (purple staining

In previous work, we identified a secreted chorismate mutase from *G. pallida*.

Similar proteins have been identified in other cyst nematodes and in root knot nematodes, suggesting that their functional role is important for a range of endoparasitic nematodes.

The *G. pallida* gene encoding the CM is expressed only in the subventral gland cells (Figure 3)

The *G. pallida* gene is expressed in J2 and in young female nematodes but is not expressed in older female nematodes

These data suggest a role for the chorismate mutase in the early stages of the host parasite interaction

Investigation of chorismate mutase function using RNAi

Second stage juveniles of *G. pallida* can be induced to take up double stranded RNA (dsRNA) from solution by incubation in the neurotransmitter octopamine

Exposure to dsRNA results in degradation of endogenous mRNAs that share the same sequence as the dsRNA. This RNA interference (RNAi) procedure allows the effects of removing a gene from an organism to be examined and allows detailed investigation of gene function

We have used RNAi with a dsRNA targeting a conserved region of the gene to examine the function of the *G. pallida* chorismate mutase

Figure 6. Uptake of fluorescently labelled dsRNA by a second stage juvenile of G. pallida

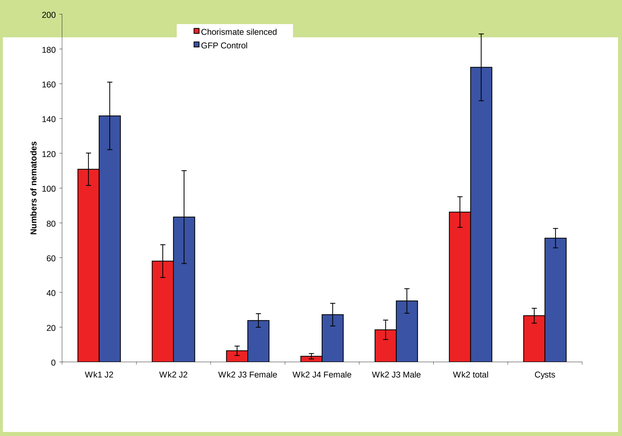

Figure 7. Developmental stages of G. pallida present on/on roots after treatment with GFP or CM dsRNA

Nematodes were exposed to dsRNA from chorismate mutase or a non-endogenous gene (GFP) as a control. Nematode stages present in roots 1 and 2 weeks after infection were counted. Numbers developing to cysts were also examined

Lower numbers of nematodes treated with CM dsRNA were present at all times compared to nematodes exposed to GFP dsRNA. The differences were greatest when comparing J4 female nematodes

Since sex is determined by environment, and specifically by food availability, in PCN this implies that exposure to CM dsRNA compromises the ability of the nematodes to induce healthy syncytia

This implies that the CM is important either in induction of the syncytium or in protecting it from plant defences

Subcellular localisation in plants

It is thought that the *G. pallida* chorismate mutase is secreted into the plant cell by the nematode

Plant chorismate mutases may operate within the plastid or in the cytoplasm. Understanding which compartment of the plant cell the nematode protein is targeted to may provide information about the function of the protein

The *G. pallida* chorismate mutase was therefore cloned as a translational fusion with the green flourescent protein (GFP) into the genome of a plant virus - TMV (Figure 4)

Viral RNA was transcribed in vitro and used to infect plants and the subcellular localisation of the GFP-CM fusion protein was examined using a confocal microscope

These experiments revealed that the *G. pallida* chorismate mutase is localised in the cytoplasm of plant cells (Figure 5)

Figure 4. Schematic diagram of virus vector used for examining subcellular localisation of the G. pallida chorismate mutase

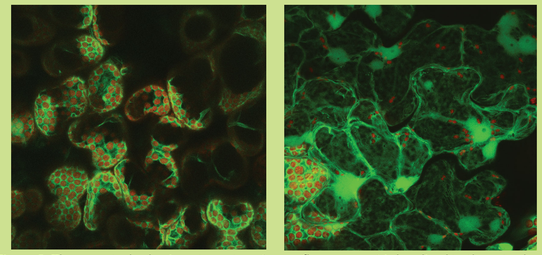

Figure 5. The nematode chorismate mutase (green fluorescence) is localised to the cytoplasm of plant cells when expressed as a fusion with GFP. Autofluorescence from chloroplasts is red.

Chorismate mutase Single Nucleotide polymorphisms are linked to virulence

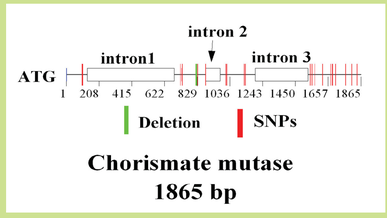

Figure 8. Schematic diagram of variation in chorismate sequences from various populatinos of G. pallida selected for virulence against S. vernei

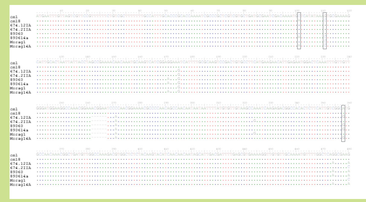

Figure 9. Alignment of part of the chorismate sequences from various G. pallida populations. SNPs studied using pyrosequencing are indicated with asterisks

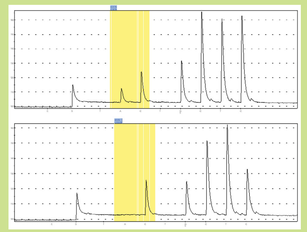

Figure 10. Output from Pyrosequencing showing differences in the proportion of an "A" residue at one SNP site between two populations

Many different copies of the chorismate mutase are present in *G. pallida*. We have examined variation within individual chorismate mutase alleles between *G. pallida* populations selected for virulence against quantitative resistance derived from *Solanum vernei*

High levels of variation were observed when comparing chorismate mutase alleles from the various populations, including deletions and single nucleotide polymorphisms (Figures 8 & 9)

We used DNA Pyrosequencing in order to obtain quantitative information about the relative proportions of each base at sites where SNPs were identified. These figures were obtained for each of the selected nematode lines that were used in this study (Figure 10)

This information was then analysed to see whether the presence of a particular base at a SNP site was linked with the virulence of the population against the *S. vernei* resistance source

This analysis allowed us to identify one SNP in which presence of one nucleotide was correlated with virulence (Figure 11)

This data provides the first genetic evidence supporting the suggestion that a particular allele of chorismate mutase may be an avirulence gene in *G. pallida*

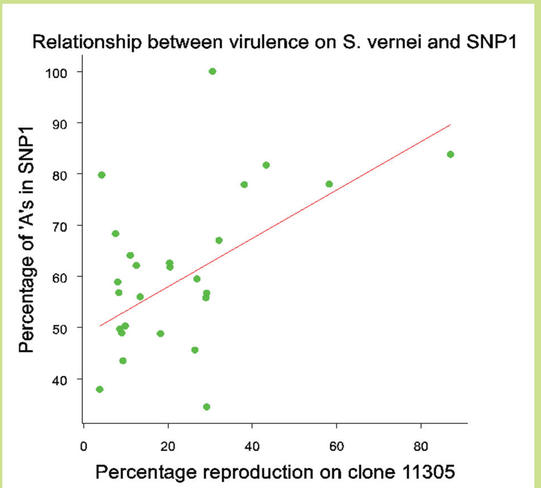

Figure 11. Presence of an "A" residue at one SNP site is correlated with virulence against resistance from S. vernei present in breeding clone 11305

Future work & Acknowledgements

Future work will include:

More detailed analysis of the role of the chorismate mutase in plants using various tools to express the protein in plant cells

Expression of the two isoforms of chorismate mutase containing the SNPs associated with virulence in plants and analysis of their relative capacity to provoke a resistant response

This work was funded by SEERAD