Pathology

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In the year of the completion of the human and Arabidopsis genomes, great strides have being made in characterising the genomes of plant pathogens and pests and the interactions of plants with their pathogens and pests. Characterisation of the genomes of bacteria, fungi, and nematodes at SCRI has yielded some exciting results, with more to follow. With viruses, where the genomes are much smaller and were characterised some years ago, research has already moved into the area of functional genomics. With the new Institute focus on (functional) genomics, proteomics, bioinformatics, and metabolic profiling, Pathology has found a new niche, allowing new interactions with other research groups at the SCRI. This means we need to reduce our efforts in some more traditional aspects of agriculture over the next few years. However, at present, we are still heavily involved in a number of projects under the broad headings of Aetiology & Epidemiology, Microbial Variation & Diagnostics, and Development of Disease Control Measures. In the last year, we have made substantial progress in all these areas but, for this Annual Report, we will highlight our achievements in the areas of Microbial Genomics and Plant-Pathogen/Pest Interactions. The struggle against plant disease continues on several fronts.

Microbial Genomics

A physical map of the genome of *Erwinia carotovora* subspecies *atroseptica* (*Eca*) is 90% completed, with over 130 genes mapped. Good progress has been made in mapping the genome of *Erwinia carotovora* subspecies *carotovora* (*Ecc*). A number of these genes are novel and may have an important role in the pathogenicity or host range of this organism.

A comparison of the *Eca* and *Ecc* physical maps shows that the genomic organisation of the two subspecies is very similar, although the positions of some genes differ between the genomes, which may explain differences in pathogenicity and host range between these pathogens.

Sample sequencing of two *Eca* bacterial artificial chromosome (BAC) clones has demonstrated major alterations in the ordering of conserved genes in the *Eca* genome vis-à-vis *E. coli*.

Sample sequencing of two *Eca* BAC clones has identified a number of novel genes that may help *Eca* survive in the soil or *in planta*. These include sequences similar to opine catabolism genes from *Rhizobacteria*, regulatory genes from *E. chrysanthemi* and sequences similar to genes from an operon required for the attachment of *Agrobacterium* to host cells.

End sequencing of *Eca* BAC clones has identified sequences that may have a role in pathogenesis. These include sequences similar to toxin genes in

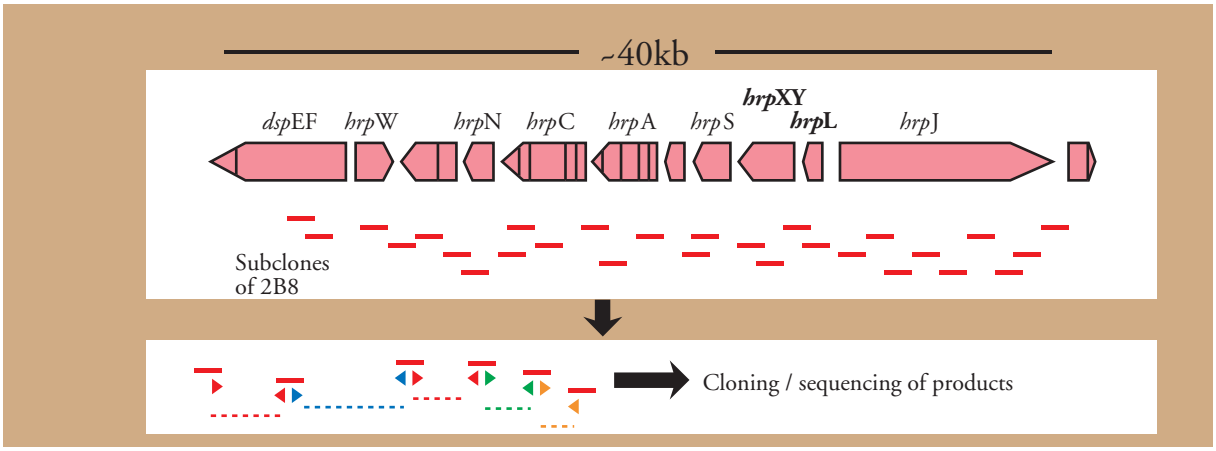

Figure 1 A number of novel pathogenicity- and host range-related genes have been identified in *Erwinia carotovora* subsp. *atroseptica (Eca)*. A Bacterial Artificial Chromosome library has been used to locate the position of an entire *hrp* gene cluster (approx. 40 kb). The cluster, thought to be responsible for the export of avirulence genes, has been sequenced using a combination of random sequencing and PCR. Phenotypic tests suggest that the cluster is functional in *Eca*.

Pseudomonas syringae and genes involved in iron scavenging in *P. aeruginosa*. Important genetic determinants of pathogenesis in *Eca*, some with analogues in animal pathogens, have been identified and characterised. An intact Type III secretion system (*hrp* gene cluster) has been fully sequenced (Fig. 1); it is very similar to the *hrp* gene cluster in *E. amylovora*, and its functionality has been confirmed by the export of the *avrB* gene in transformed *P. syringae*. Two more *E. amylovora* disease-specific genes adjoin it. This finding is surprising, as pathogenesis in these two species is very different. The *hrp* gene cluster may have some hitherto unforeseen role in the early stages of infection by *Eca*.

Sequences also have been identified in *Eca* that are homologous to haemolysins in various animal pathogens. Although some are similar to the *hecAB* genes from *E. chrysanthemi,* others have no similarity to any known *Erwinia* genes. Haemolysin homologues are present in various other plant pathogens and have a role in attachment of *P. putida* to plant seeds.

A number of important proteins and/or genes encoding such proteins have been identified associated with the potato cyst nematode (PCN). These include the following:

A gene for pectate lyase, encoding an enzyme that probably aids in the penetration of the host by the nematode. This is the first report of a pectolytic enzyme in any animal.

A peroxiredoxin, a major hydrogen peroxide removal enzyme, is located on the surface of PCN. Similar peroxidases in nematode parasites of animals are involved in cuticle cross-linking and in protecting the nematode from superoxides generated by the host's defence mechanisms.

A family of chitin synthase genes. One gene product is involved in the synthesis of eggshells and another in part of the nematode feeding apparatus. Also identified was a subgroup of collagen genes responsible for fuelling the cuticle growth of adult nematodes.

A novel family of nematode secreted proteins has been characterised and the proteins have been shown to be expressed in different nematode secretory tissues, implying different functional roles in the development of PCN.

In viral genomics and functional genomics, there also have been a number of advancements.

Raspberry bushy dwarf virus (RBDV, genus *Idaeovirus*) has a bipartite genome that consists of a *c.* 5.4kb RNA-1 and a *c.* 2.2kb RNA-2. RNA-1 encodes the proteins that are involved in virus replication. RNA transcripts from full-length cDNA clones of each of the genome RNAs of RBDV were shown to be infective, opening up the possibility of reverse genetics, to determine the nature of resistance breakage by the RB isolate.

The cell-to-cell movement protein (MP) of *Groundnut rosette virus* (GRV), encoded by ORF4, was expressed from *E. coli* and affinity purified. Gel retardation analysis demonstrated that in contrast to many other viral MPs, including the 3a MP of *Cucumber mosaic virus* (CMV), the ORF4-encoded protein bound to viral single stranded (ss) RNA non-

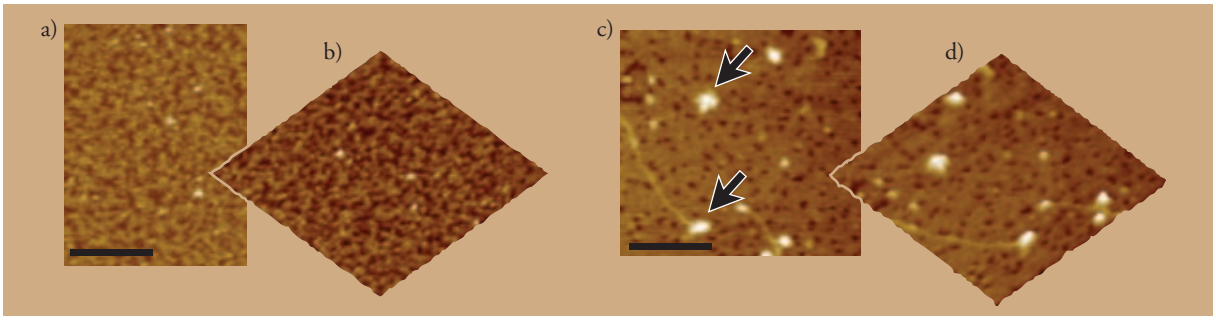

Figure 2 Atomic force microscopy (AFM) allows three-dimensional imaging and measuring of individual biomolecules in the nanometre range under ambient and physiological conditions. AFM was used to characterise the architecture of complexes formed *in vitro* by viral RNA and movement protein (MP) encoded by two different plant viruses, *Cucumber mosaic virus* (CMV) and *Groundnut rosette virus* GRV). Analysis of CMV MP-RNA complexes revealed chains of CMV MP molecules, presumably bound to RNA (a, b). By contrast, GRV MP formed unexpected structures containing chains of ribonucleoprotein extending from large globules (arrows) (c,d). Bars = 300 nm.

cooperatively and formed complexes of low protein:RNA ratio. UV crosslinking and nitrocellulose membrane retention assays confirmed that the both GRV MP and CMV MP formed complexes with ssRNA and the complexes were of similar stability. Probing MP-RNA complexes with atomic force microscopy demonstrated that the ORF4-encoded protein bound to limited regions of viral RNA while the CMV 3a protein formed highly packed complexes (Fig. 2).

The GRV ORF4-encoded protein was also shown by immunofluorescence microscopy to generate tubular structures protruding from the protoplast surface. Such tubules are also produced by the CMV MP.

Nucleotide sequences of the cylindrical inclusion (*CI*) gene of 15 isolates of *Potato virus Y* (PVY) showed there to be greater diversity between strain sub-groups than was previously supposed. There seem to be two classes of CI sequence that are characteristic of the two main PVY sub-groups, but there is also evidence that recombination has occurred between viruses in different sub-groups.

Preparations of *Tobacco rattle virus* (TRV) of sufficient concentration and purity for measurement of Raman optical activity were made. Studies on the TRV preparation (collaboration with L. Barron, University of Glasgow) provided experimental support for a previous theoretical model of TRV particle structure, but with some additional details.

A self-interaction was demonstrated in the yeast two-hybrid system by protein p51 encoded by RNA-2 of *Potato mop-top virus* (PMTV). Furthermore, the protein was shown to bind ssRNA. This work indicates that p51 functions to transport RNA from cell to cell during the virus movement process.

The PMTV RNA-2 proteins p21 and p13 were expressed as fusion proteins with GFP from the TMV viral vector. Localisation of p21-GFP by confocal laser scanning microscopy indicated that it accumulated at or near plasmodesmata, whereas p13-GFP appeared as small aggregates in the cytoplasm. No interaction was found between p13 and p21 in the yeast two-hybrid system. This work will lead to the development of a model for virus protein function in virus movement.

Plant-Pathogen/Pest Interactions

Many genes that are important in early cell signalling, plant defence and response to stress, have been isolated from potato after challenge with pathogenic bacteria, fungi and nematodes.

Using RT-PCR (TaqMan), initial experiments have been carried out quantifying expression analyses of potential signalling and response genes, including a protein phosphatase (PP2A) regulatory subunit, a TATA-binding associated factor, a receptor-like kinase, and a ubiquitin-specific protease. Expressed sequence tags (ESTs) for these genes had been obtained from a suppression subtractive hybridisation (SSH)-derived library of potato genes up-regulated 1 hour after inoculation of potato leaves with *Eca*.

A potato gene, *erg-1* (Erwinia-response gene), is rapidly induced by *Eca*, *Phytophthora infestans*, ethylene and salicylic acid. This gene is a member of a family of related stress-responsive genes of unknown function.

A potato gene encoding a WRKY-like transcription factor was shown to be induced in interactions with *Eca* and *P. infestans* and to be co-regulated with a class 1 endochitinase. Sequences of the 5' untranslated region (UTR) for St-WRKY-1 and the endochitinase

were determined and found to contain a number of potential binding sites for cis-regulatory elements. The 5'UTR of the chitinase contained potential W-box WRKY-binding regions.

SSH has identified genes expressed in tomato roots infected with *Meloidogyne chitwoodi*. Genes previously associated with stress, infection and apoptosis were found, including the *Le Mir* gene, proteases, pathogen related proteins, a nodulin homologue and the resistance gene *Mi-1*. A similar approach with compatible and incompatible plant PCN interactions has generated an EST library, unexpectedly rich (30 - 50%) in nematode sequences. cDNA-AFLP of plant-PCN interactions has also identified a large number of plant genes putatively involved in the host response, including three NBS-LRR genes not currently in public databases.

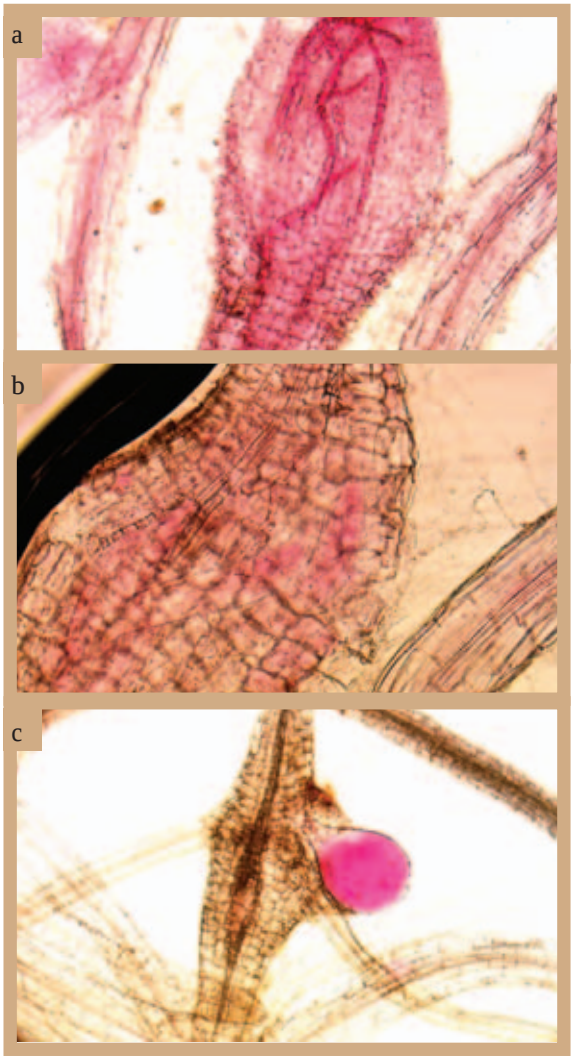

Figure 3 The time course of *Meloidogyne chitwoodi* parasitising the resistant potato, *S. bulbocastanum*. (A) At 3 days post infection, second stage juveniles (J2s) invading the roots are seen moving intercellularly and orientating themselves in the root tips before migrating to the vascular cylinder. (B) At 10 days post infection, the nematode, having established a feeding site, is seen enlarging and moulting. Oesophageal gland secretions have altered the expression of host genes and multinucleate 'giant cells' form the established feeding site. Characteristic swelling of the host plant cortical cells has occurred (cf. adjacent, unthickened, normal root). (C) At 21 days post infection, after moulting three times, the adult, female nematode is seen breaking through the cortex of the root.

A histological examination of nematode development in Pentland Ivory (susceptible to PCN (*Globodera pallida*) and potato clone 12601ab1 (with PCN resistance derived from *Solanum tuberosum* spp. *andigena* CPC2802) has been made. Nematode development is retarded in 12601ab1 relative to Pentland Ivory, which is consistent with the typical delayed resistant response for cyst nematodes. A histological examination of *Meloidogyne chitwoodi* (Virulent line V6) successfully parasitising the resistant potato *S. bulbocastanum* is shown in Figure 3.

SSH was used to discover genes involved in compatible (virulent) and incompatible (avirulent) challenges by *Rhynchosporium secalis* in barley. Several novel and resistance-related genes were identified and their regulation is being characterised.

The *Hero* gene is a major wide-spectrum disease resistance gene of tomato that confers resistance to PCN. It has been isolated by a map-based cloning method in a close collaboration with a German research group. PCN-susceptible tomato plants, became resistant to PCN after transformation with the *Hero* gene. This provides an excellent opportunity to dissect the molecular mechanisms of PCN resistance in plants and to exploit this useful gene to develop PCN resistant potato cultivars.

Virus resistance responses have been characterised in several host-derived and pathogen-derived (transgenic) systems.

Simultaneous expression in tobacco of transgenes encoding sense and antisense sequences of the *CI* gene obtained from a PVY^{O} sub-group isolate, gave very strong resistance to other isolates of PVY^{O} but not to isolates from the PVY^{N} subgroup.

Plants of the *S. tuberosum* clone G8107(1) were found to be immune to infection with *Potato leafroll virus* (PLRV) following inoculation with very large numbers of viruliferous aphids, although the plants were readily infected by grafting. Results of sensitive detec-

tion tests on inoculated plants suggest that virus delivered by aphid inoculation is unable to exit the inoculated leaves. This type of resistance has not been identified in other lines of *S. tuberosum*.

Resistance to CMV in CMV RNA-1 transgenic plants was found to be sequence specific but not to be caused by post-transcriptional gene silencing. Resistance to both long-distance movement of CMV and replication of CMV RNA-1 could be overcome by graft inoculation, suggesting the presence of a component of the resistance mechanism that could be saturated.

Specific topics not covered above are given in more detail in the following articles.