Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP - 2006/2007 SEASON

Project title Molecular analysis of pathogen-cotton interactions

Aims and milestones
The main aim of this summer studentship was to train Ms Rebecca Forbes in general and molecular techniques required for dealing with *T. basicola* handling and mutagenesis. The skills she would learn and develop during the summer studentship and the preliminary experiments would serve her and other members of the group in future experimentation with this fungal pathogen.

Milestones included: (1) learning the rules and general behaviour in a PC2 laboratory (2) learning sterile techniques (3) learning how to grow and handle the fungal pathogen and other microorganisms needed in the project (4) learning the skills of medium, solution and buffer preparations (5) performing transformation and electroporation of Agrobacterium for ATMT and of E. coli for plasmid preparation (6) attempt current protocols for ATMT. All milestones had been achieved by the end of the summer studentship and the student continued into an Honours degree in 2007.

Staff: Ms. Rebecca Forbes (Supervisor Dr Lily Pereg-Gerk)

Project Summary

Background
Black root rot is recognised as a threat to the cotton industry, causing up to 26% yield loss. Current management strategies are insufficient for disease control and further research is required in order to develop new strategies to reduce the impact of the disease on the cotton industry. To achieve this, we must first gain understanding of the steps crucial for completing the life cycle of the pathogen, T. basicola, to be able to control the disease in a sustainable manner, i.e. with reduced input, increased profit and reduced negative environmental impact. One of the most important factors in the plant-pathogen interactions is their initial communication via the exchange of signal molecules. By understanding T. basicola-cotton communication signals, and the reaction of both organisms to such signals, we can develop strategies to interfere with their association and, thus, reduce the events of the black root rot disease in cotton. This project relates to the CRC's sub-program: The Farm, as it has the potential to develop improved integrated management systems for cotton diseases that are profitable, sustainable, with strategies developed for plant breeding/engineering against black root rot, less reliant on input of anti-fungal substances and thus promotes soil health.

Aims and objectives
The main aim of the overall project run in our lab is to develop a range of tools for studying the molecular factors involved in the initiation and establishment of cotton infection by *T. basicola*. We intend to use these molecular genetics and proteomics methodologies as well as plant-pathogen assays in an extensive study of genes and proteins, involved in *T. basicola*

pathogenicity towards cotton and the host response to fungal infection. By understanding *T. basicola*-cotton communication signals, we can develop strategies to interfere with their association and, thus, reduce the events of the black root rot disease in cotton.

The objective of this summer studentship is to train Ms Rebecca Forbes to become one of research group, particularly concentrating on molecular genetics techniques to study pathogenicity genes of *T. basicola*. This included preliminary experiments in Agrobacterium-mediated transformation (ATMT) of *T. basicola*.

Methodology and Results

General Laboratory Skills and Training in Equipment

Ms Rebecca Forbes received training in the requirements of proper conduct in a PC2 laboratory early in the studentship. This ranged from the requirements of how to dress appropriately (i.e. always wear a lab coat and closed shoes) to more specific requirements on how to contain the genetically modified and pathogenic organisms being dealt with in the lab on a daily basis.

In addition, she learnt how to safely and effectively use a number of laboratory equipment including the autoclave, electropulse machine, and UV transilluminator.

Handling of *T. basicola* & *A. tumafaciens*

As *T. basicola* was one of the main organisms that Rebecca would be dealing with extensively throughout her Honours year, effective methods in the handling, growing, etc of this organism were learned from the first day that she entered the lab. She first learned the location in the lab where work with this fungus was to be conducted, namely the UV PC2 hood, and how to ensure that the hood was maintained in a sterile condition at all times. Following this, she was introduced to a number of methods involved in handling and preparing *T. basicola*. This included how to prepare spore suspensions of the endoconidial cells, preparation of streak and stab plates for growth of the fungus, and the long-term storage of *T. basicola* as agar blocks.

She also was trained in how to perform pathogenicity tests with wild type and mutant strains of *T. basicola* in order to assess the level of disease symptoms on cotton seedlings. As well, she was taught how to do directional growth tests with *T. basicola* (wild type and mutants) to determine its growth towards the cotton seedlings.

Additionally, she learned how to extract and prepare genomic DNA from *T. basicola*. She performed this method under supervision of one the PhD students as she was preparing fungal DNA in preparation for a Southern Blot analysis.

She also learned how to handle and grow *A. tumefaciens* (AGL1) as this is the second major microorganism that she would be dealing with in her Honours project. She learned, through a number of trial-and-error experimentations, the timing and particular techniques required to grow this apparently sensitive organism.

Competent Cells

Following protocols provided, Rebecca became skilled in preparation of $CaCl_2$ competent *E. coli* DH5α cells for subsequent use in heat shock transformations. Also, the preparation of electroporation-competent *A. tumefaciens* cells for subsequent use in electroshock transformations.

Preparation of Media and Media-Related Solutions

Rebecca learned how to prepare media and related solutions commonly used to cultivate the organisms that she would be working with during her Honours, including:
YT, LB, ½ PDA, PDB, IM, IMAS, M-100, M-100 Trace Element Solution, M-100 Salt Solution, MES, MM Salts, YMA, Acetosyringone, SOB, and SOC.
In addition, she learnt how to prepare numerous different solutions used for a range of general and specific purposes in the lab. As well, she was taught how to prepare sterile stock concentrations of antibiotics, including ampicillin, kanamycin, hygromycin, chloramphenicol and Mefoxin; all of which would use extensively throughout her project for selective purposes.

Transformations

After preparing competent cells, Rebecca practiced heat shock transformations of *E. coli* DH5α cells, in which she generated successful transformants with the plasmids pBHt2, pBR322, and pGpdGfp. Also, she practiced electroporation transformations of *Agrobacterium* cells, in which she generated successful transformants with the plasmid pBHt2 (the key plasmid that she is using at present in her Honours) and more recently with pPPK2 (a second plasmid that she is working with).

Mini-Preparations of Plasmid DNA

From all transformants that Rebecca prepared, she learnt how to recover the plasmid DNA (either for further confirmation of successful transformants, in addition to antibiotic selection, or to be able to use in her later experiments), from the cells using a standard mini-preparation.

Gel Electrophoresis

During this time Rebecca practiced the preparation of mini-gels, both in standard gel equipment as well as the more difficult technique on glass plates. She practiced running a number of different plasmids and DNA fragments on the gels, learning which concentrations of agarose and DNA produced the highest resolution in differing circumstances. From these gels she also learnt how to confirm the correct plasmid, estimate DNA sizes and concentrations, and extract and purify DNA fragments for subsequent ligation experiments.

Restriction Enzyme Digestions

Rebecca gained knowledge in how to perform a number of RE digestions on several plasmids; learning which enzymes to choose and under which conditions (e.g. buffer, incubation temperatures, etc.) they worked best (especially for double digests). Some of the RE digestions she has performed included (1) pGpdGfp with KpnI and NotI, (2) pBR322 with EcoRI, BsaAI, and EcoRI & BSaAI, and (3) pBHt2 with KpnI, EcoRI, SmaI, NheI, EcoRI & SmaI, EcoRI & NheI and SmaI & NheI.

Ligations

Rebecca practiced how to perform ligation reactions to combine fragments from several different plasmids. She performed a 2-piece ligation between pBR322 (EcoRI & BSaAI) + pBHt2 (EcoRI & SmaI) fragments as well as a 3-piece ligation between pBR322 (EcoRI & BSaAI) + pBHt2 (EcoRI & NheI) + (SmaI & NheI) fragments.

ATMT

Finally, Rebecca tried her hand at performing several *Agrobacterium*-mediated transformations of *T. basicola* with *Agrobacterium* [pBHt2]. During this time, she learnt the timings required for the growth of *T. basicola* and *Agrobacterium* both prior and immediate growth times. She learnt how to effectively grow these organisms together and explored some of the various interactions that seem to occur between them. In general, she got a very good feel of how to perform ATMT according to this particular protocol, which was helpful for her subsequent ATMT tests that she has been performing throughout her Honours project so far.

Conclusion
Ms Rebecca Forbes performed 8-week summer studentship at the University of New England in December 2006 - January 2007. Her scholarship for this studentship was kindly provided by the CCC-CRC.

During these 8 weeks she learnt numerous methods and techniques ranging from general laboratory skills to more specific techniques in preparation for her Honours degree that commenced directly after the studentship in February 2007.

Presentations and public relations
Rebecca will present her project in the CCC-CRC meeting, Narrabri, 8-9th August 2007.