



Cotton Catchment Communities CRC

SUMMER SCHOLARSHIP - 2007/2008 SEASON

Project 5.10.03.16:

Project title Molecular factors in pathogen-cotton interactions leading to black root rot

Aims and milestones

The main aim of this summer studentship was to introduce Ms Anna Balzer, a second year undergraduate student, to research in cotton pathology, in particular how to develop research strategies and how to conduct experimental work on the cotton pathogen *T. basicola*. The training included general and molecular techniques for working with the fungus and the plant. She learned and developed useful skills for fungal research during the summer studentship and the findings in the preliminary experiments she conducted serve other members of the group in working 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 as well as adopting and optimising a suitable minimal medium for *T. basicola* (5) performing transformation of *E. coli* for plasmid preparation (6) phenotypical analyses of *T. basicola* pathogenicity mutants and (7) preliminary analysis of protease excretion by *T. basicola* (8) experience work on black root rot in cotton soil growth-system under laboratory conditions. All milestones had been achieved by the end of the summer studentship and the student continued with her undergraduate degree (3rd year).

Staff: Ms. Anna Balzer (Supervisor Dr Lily Pereg-Gerk)

Project Summary

Black root rot is a recognised threat to the cotton industry, yet current management strategies are insufficient for disease control. Thus, 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.

Understanding the interactions between the pathogen and the plant requires a multidiscipline study involving collaborations among scientists expert in research fields such as microbiology, plant biology, soil biology, fungal genetics, molecular biology and agronomy. It is extremely important to train and educate students in these fields towards understanding plant disease and its management.

The summer student Ms Anna Balzer, got intensive training in molecular biology and

microbiology, particularly in handling the fungal pathogen *T. basicola*, and in conducting pathogenicity assays to assess black root rot in cotton. She also conducted assays to examine pathogenicity-related properties in *T. basicola*, to further our understanding of factors involved in its virulence towards plants. One of the highlights from the results is the optimisation of a minimal growth medium for the fungus, which will facilitate further studies of pathogenicity related traits of *T. basicola*.

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 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 Anna Balzer to learn about research practicalities, particularly concentrating on general and molecular genetics techniques to study cotton pathogens.

The following is a summary by Ms Anna Balzer of her summer studentship experience:

Cotton CRC Summer Studentship, by Anna Balzer

During my eight weeks work experience in a research lab I gained many valuable skills and a great deal of knowledge. The practical skills I gained, including media preparation, aseptic technique, microscopy, photography, transformations, DNA extractions and fungal transfers among others, are often not provided in a typical practical session with the units I undertake through the university. All these skills are likely to prove useful in potential careers, workplaces and in prospective postgraduate studies. Being able to experience these first hand and to be involved in them is a much greater learning tool than simply reading about or being told of these methods. The first hand experience will hopefully allow me to understand in greater depth many of the scientific methods I am likely to encounter during my third year studies. In addition, I was exposed first hand to the processes involved in research in general and also learned a great deal about the fungal pathogen *Thielaviopsis basicola*, its' impact on the cotton industry and some of the research being undertaken in attempt to control its detrimental effects, including identification of pathogenicity genes and biological control measures.

Over the past eight weeks it has become evident to me that in research situations, experiments often do not succeed the first time and procedures are constantly being modified to provide the ideal conditions for their specific purpose. It has also made me aware that for every experiment that does not work, there is always the opportunity to repeat the procedure in an attempt to improve its' outcome and efficiency. Experiments can also provide unexpected results, leading to further experiments, hypotheses and ultimately answers.

The first four weeks of my time in the laboratory were used to familiarise myself with common practices, PC2 training, learning about *T. basicola* and the research which is being undertaken in relation to it at UNE. This continued during the last four weeks of my time there, however, I was also encouraged to undertake a small project. The project involved the growth of *T. basicola* on various media, including a protein source in an attempt to determine whether *T. basicola* exhibited extracellular protease activity and under what conditions this may occur.

Some of the media tested included rich media with protein sources and media prepared to imitate nutrient deficiencies of carbon and nitrogen. This process entailed searching literature for a minimal media suitable for *T. basicola*, and modifying it to suit the experimental design. The media proved successful, however may potentially be improved by substitution with an alternative nitrogen source, which is more carbon minimal than the one suggested by the literature. The results of the experiments appeared to show that wild type *T. basicola*, and five mutant strains of the fungus showed little or no extracellular protease activity under the conditions tested. In addition I carried out soil pathogenicity assays of the wild type and mutant strains of *T. basicola*. This test appeared to suggest that several of the mutant strains, with disrupted genes showed a lower pathogenicity toward cotton, potentially indicating that the disrupted genes are associated with plant pathogenicity.

The opportunity to undertake this work experience has been an incredibly valuable learning tool for me and has certainly drawn my attention to research and the prospect of furthering my studies including an honours year.

Methodology and Results

General Laboratory Skills and Training in Equipment

Ms Anna Balzer 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, centrifuges, and UV transilluminator.

Handling of *T. basicola* & *E. coli*

Effective methods in the handling, growing, etc of *T. basicola* were learned throughout the 8 weeks of the studentship. 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. In addition, she was shown how to conduct pathogenicity tests with *T. basicola* (wild type and mutants) in soil system.

Additionally, she was shown how to extract and prepare genomic DNA from *T. basicola*.

E. coli Competent Cells

Following protocols provided, Anna became skilled in preparation of CaCl₂ competent *E. coli* DH5α cells for subsequent use in heat shock transformations.

Preparation of Media and Solutions and pH adjustment

Anna learned how to prepare media and related solutions commonly used to cultivate microorganisms, including: YT, LB, ½ PDA, and PDB as well as to adjust the pH to the optimal growth conditions for each microorganism.

In addition, she learnt how to prepare numerous different solutions used for a range of general and specific purposes in the lab. She was also taught how to prepare sterile stock concentrations of antibiotics, including ampicillin, kanamycin and hygromycin, all of which would use extensively throughout her project for selective purposes.

Transformation of E. coli

After preparing competent cells, Anna practiced heat shock transformations of *E. coli* DH5α cells, in which she generated successful transformants with a lacZ containing plasmid. She learned blue/white selection of lacZ-transformed *E. coli* colonies.

Gel Electrophoresis

During her studentship Anna practiced the preparation of mini-gels. 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.

Soil and agar techniques for T. basicola pathogenicity tests

Anna observes and conducted pathogenicity tests to assess the level of black root rot on cotton. She used two common techniques in our lab – the agar plate system for fast results and the confirmation technique using the soil system.

Minimal medium development for T. basicola and protease studies

Anna not only gained technical skills but also trained in developing research strategies and conducted a small project that proved beneficial for the lab. Her task was to search the literature for fungal minimal media and adopt as well as optimise a medium suitable for *T. basicola*. She did so successfully, to the benefit of the entire group.

She also participated in literature search and conducting experiments to investigate protease secretion by *T. basicola*. Although the results of the experiments were not conclusive regarding protease excretion, the experiment results and design proved useful as they are of technical values for the optimisation of future experiments.

Conclusion

Ms Anna Balzer performed 8-week summer studentship at the University of New England in December 2007 - February 2008. 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 for studying fungal disease in cotton. She was trained in designing and performing scientific research.

Presentations and public relations

Anna was interviewed and photographed for an article which appeared on the UNE website regarding summer studentship provided by the CCC-CRC. She also featured in UNE's Presentation Ceremony for scholarship holders, and was photographed with the CRC Chief Scientist.