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Development of Methods allowing Correlation of *Dothistroma* and Dothistromin *In Planta*

A thesis presented in partial fulfilment of the
requirements for the degree of

Master of Science
in
Biochemistry

at Massey University, Manawatu,
New Zealand

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2010

Abstract

Dothistroma septosporum is a fungal pathogen of pines with a worldwide distribution. It is responsible for the disease red band needle blight, in which necrotic lesions appear on infected needles. The red colour of the disease is due to the presence of the mycotoxin dothistromin. This toxin is structurally related to the better characterised mycotoxins aflatoxin and sterigmatocystin. The function of these toxins is unknown, but dothistromin is hypothesised to act as a competition factor. While much work has been done on *D. septosporum* and dothistromin in broth culture, *in planta* work has been limited by the methods available.

This work focused first on the development of a method for the reliable and high yield extraction of DNA from infected lesions, as previously used methods were found to be inadequate. It was found that the addition of an enzyme lysis step to the Qiagen DNeasy protocol and the replacement of its column purification with chloroform purification gave a greatly increased yield of DNA with an acceptable loss of purity.

To allow quantification of dothistromin from the same lesion samples, previously used assay systems were optimised and compared in their accuracy and sensitivity. An HPLC-fluorescence method was found most effective, and was able to accurately quantify dothistromin at single lesion quantities.

The developed methods were used to give a correlation between *Dothistroma* biomass and dothistromin in lesions at various stages of development. While this correlation was not found to be statistically significant, continuation of this work should allow valid conclusions to be drawn.

To give insight into the evolution of dothistromin biosynthesis, the genomes of other dothideomycetes were examined for the presence of dothistromin biosynthesis gene homologs. While no homologs were conclusively identified, a number of genes were shown to have similarity to known toxin biosynthesis genes.

In summary, while not all research hypotheses were able to be proven or disproven, this work sets a firm basis for future investigation in these areas using the methods developed, and strongly suggests the direction continued study should take.

Acknowledgements

First and foremost, my sincere thanks to my supervisor, Rosie Bradshaw, not only for giving so much guidance and support in my work, but also for putting up with my constant misuse of the word ‘significantly’.

Thank you to Rose Motion, for giving up so much of your time to help me with the chemistry side of things, of which I still know far too little about.

Thank you to the past and present members of the Fungal Jungle who have been so helpful; Carole, for always knowing where everything is, especially if it’s right in front of me; Arne, for pointing me in the right direction; Rebecca, for running the real time PCR and always having such good advice; Shuguang, for showing me through the methods; Melissa, for showing that there is light at the end of the tunnel; Kabir, for all his help with the pine seedling work; and to Adam, Shuiying, Yanfei, and Pranav for answering my mostly inane questions.

Thank you to everyone from IMBS for being so friendly and so willing to give up your time to help someone else. Unfortunately there is no way I can name all of you!

Thanks to Robert and Rex for giving me excellent advice on working with dothistromin. It was a privilege to have benefit of such experience. Thanks to Karl and Scott of AgResearch for the MS work.

More personally, thanks Kerry for being amazing, and thanks Mum and Dad for all the support.

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