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***CLONING AND CHARACTERISATION OF TWO
SUBTILISIN-LIKE PROTEASE GENES FROM
NEOTYPHODIUM LOLII***

A Thesis presented in partial fulfilment of the
requirements for the degree of
Master of Science in Molecular Genetics
at Massey University, Palmerston North,
New Zealand

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2000

ABSTRACT

PCR amplification of *Neotyphodium lolii* genomic DNA with degenerate primers detected two different sequences with homology to subtilisin-like proteases. These two PCR products were used to screen a *N. lolii* Lp19 genomic library.

The *prt1* gene was isolated by screening the genomic library with the GH30 PCR product. This gene encodes a putative peptide of 434 amino acids that is most similar to subtilisin-like proteases from *Aspergillus* sp. The *prt1* gene contained a single intron, which was in a position conserved with other fungal genes. 3'RACE was used to determine the polyadenylation site for the *prt1* gene.

Repetitive DNA was a feature of both the 3' untranslated region (UTR) and sequences downstream of the *prt1* gene. Within the 3' UTR, a complex microsatellite was found extending over 50 base pairs. Downstream of the gene, a minisatellite locus of 360 base pairs in size was found, consisting of 40 copies of a 9 base pair AT-rich repeat.

Expression of *prt1* was examined in cultures with various types of carbon and nitrogen sources. Although no conclusive results could be drawn, the type of carbon and nitrogen available did have some effect on *prt1* expression. Repression of *prt1* expression was only observed in media supplemented with sucrose and glutamate.

A 500 bp fragment from the *prt1* promoter was introduced into the vector pFunGus to create a translational fusion with *gusA*. This vector, pMM9, was transformed into *Penicillium paxilli*. Although transformation frequencies were low, the transformants obtained appeared to be stable for hygromycin resistance. Expression of GUS was observed in seven out of twelve of the stable transformants. This showed that the promoter fragment in pMM9 was sufficient for expression of GUS in a heterologous system.

The *pri2* gene was isolated by screening a genomic library with the GH3 PCR product. Partial sequence has been obtained for the *pri2* gene. The *pri2* gene contains at least three introns, the first of which is conserved with *pri1*. From the sequence obtained, *pri2* encodes a peptide with strong similarity to subtilisin-like proteases from *Metarhizium anisopliae*, a fungal pathogen of insects.

ACKNOWLEDGEMENTS

Firstly, I would like to express my thanks to my supervisor, Professor Barry Scott, for his excellent supervision during my project. I have deeply appreciated your support and encouragement throughout my thesis. Thanks to Dr. Grant Hotter for his initial interest and work on this project, especially for the primer design and probe preparation. To Mike Christensen, thank you for our discussions on the endophytes and their interactions with their hosts. Your enthusiasm and knowledge about endophyte associations has been an inspiration. Thanks also to Dr. Andrew Griffiths for the endophyte genomic DNA used for Southern blotting in Figures 3.2 and 4.2, and to Dr. Peter Farley for the conversations on proteases and their regulation. Thanks also to Dr. Gretchen McCaffrey for her supervision while Barry was away on sabbatical and her encouragement throughout my degree.

To Carolyn Young, how do people in other labs cope without you? I'd probably still be library screening if it wasn't for your help. Thanks for all your help and advice for the practical work, and especially for proof-reading most of this thesis and helping me to put it all together. To Lisa McMillan, thanks for your help with the proofreading, for answering all my questions and for help learning all those new experimental procedures. To my fellow lab-members past and present, Christina, Austen, Emily, Xiuwen, Renae, Raj, Rohan, Mike and Shuguang, and the other members of MGU (Bek, Beccy, Seth, Janet, Paula and Jonathan) thanks for your help and support. To Bec, thank you for your encouragement during the writing of this thesis, especially when I felt it would never be finished.

To my parents Pat and Teresa, I could never, ever have achieved this without both of you. Thank you so much for all your love and support, both financial and emotional, especially during the times I would have given up without your help. To my sister Roslyn, thanks for always being there for me, and thanks also to my brothers Steven and Desmond. Special thanks must go to my flatmates for their encouragement while I have been writing. Todd, Andrew, Larnie and Shan, thanks for being great friends. You made sure life at the flat was fun and that I didn't stress out too much. Thanks for the many laughs and songs, although I'm not sure if I want to remember all of the lyrics! Thanks also to Htar for her friendship, support and encouragement.

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