Development of an Efficient Transformation System for *Dothistromin pini*

A thesis presented in partial fulfilment
of the requirements for the degree of
Masters of Science in Molecular Genetics

at

Massey University, Palmerston North
New Zealand

Anita Therese Bidlake

1996

Abstract

A transformation system has been developed for the plant pathogenic fungus *Dothistroma pini* using a positive selection system based on the *Escherichia coli* hygromycin B phosphotransferase gene (*hph*). After optimising the conditions under which protoplasts were isolated a transformation protocol was determined. The system developed gave large, stable transformants at frequencies between 1 and 48 transformants per μg of DNA. A second type of colony also grew on the selective plates. These grew in higher numbers but less vigorously, and did not grow when subcultured onto plates containing hygromycin B. These are believed to be abotive transformants. Southern analysis indicated that transformation takes place via the integration of the plasmid DNA into the fungal chromosomal DNA. The DNA integrated at a single site in 88% of the transformants, with all of the sites containing only a single copy of pAN7-1. Propagation of two of the transformants through single spore analysis indicated that they were homokaryons, though molecular results of another transformant indicated that it was a heterokaryon. Placing the transformants on increasing concentrations of hygromycin B indicated that the copy number of the integrated plasmid was not related to hygromycin resistance. In order to try and enhance the transformation rate of *Dothistroma pini*, by using a homolgous promoter, the β-tubulin gene was isolated from a genomic library using the β-tubulin gene from *Neurospora crassa* as a probe. A restriction map was made and the gene was sequenced and shown to closely resemble β-tubulin genes from other fungi.

Acknowledgements

Firstly my sincerest appreciation must go to my supervisor Dr. Rosie Bradshaw. Rosies optimism, encouragement, patience and organisation have played a huge part in guiding me to the completion of this momentous task.

Special thanks to all the people in my lab who helped and supported me. For Carmel who got me started, Dianne who keeped me going on my transformations, and Linda and Paul who were always around for advice and a chat. Thanks also to Branwyn and Brendon who brought some new life into our lab. Brendon must also be thanked for his technical assistance along with Shalome Campbell and Tash Forester. A big thank you must also be extended to the "MGUs personal" technichan Carolyn Young. To everyone else in the MGU and the department thankyou all very much for advise and friendship, I really appreciated it.

Gratitude must then be expressed to my friends and family outside the department. Thanks for friends and flatmates who have provided an ear for listening and sources of entertainment. Thanks to my family especially Dad and Judy for giving me lots of love. And special thanks to Granny and Grandpop and their garden for keeping me healthy. A special acknowledgment must be given to someone who can not read, Squeak whose antics always keep me laughing and cuddles keep me smiling.

I would especially like to thank Paul whose love and support have been really special. Without his help this thesis would have been an even longer time coming.

Basicaly to all the lovely people who have supported me and made me laugh over this time thank you, thank you, thank you!!!

Table of Contents

LIST OF TABLES

LIST OF FIGURES