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**FUNCTIONAL ANALYSIS OF A THIAMINE
BIOSYNTHETIC GENE IN THE INTERACTION OF
EPICHLÖË TYPHINA WITH PERENNIAL RYEGRASS**

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

Doctor of Philosophy
in
Molecular Genetics

at Massey University, Palmerston North,
New Zealand

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2004

Abstract

Epichloë/Neotyphodium endophytes are a group of clavicipitaceous fungi that form symbiotic associations with temperate grasses. The asexual *N. lolii* form asymptomatic mutualistic associations with ryegrass whereas the sexual *E. typhina* behaves similar to a mutualist during the vegetative phase of plant growth but switches to epiphytic growth and formation of an external stroma upon development of the floral inflorescence. The aim of this project was to study the metabolic interaction between these endophytes and their perennial ryegrass host. The role of endophyte thiamine biosynthesis in host colonisation and stroma development was chosen, because of the key role this coenzyme plays in primary cellular metabolism and because thiamine biosynthetic genes are induced in several fungal-plant interactions.

The orthologue (*thi1*) of *Saccharomyces cerevisiae* *THI4* was isolated from *N. lolii* and *E. typhina* by PCR using degenerate primers designed to conserved regions of known thiazole biosynthetic genes. This gene is expressed *in planta* and in culture, and is alternatively spliced, with distinct patterns of the isoforms expressed under different nutritional conditions. Mutant with a deletion in the *E. typhina thi1* gene was constructed and shown to have reduced hyphal density and branching compared to the wild-type on defined media lacking thiamine. Both thiamine and thiazole complemented this defect. Artificial inoculation of the mutants into plants showed that the *thi1* mutant retained the ability to colonise the perennial ryegrass host and form stromata. However, the mutant had some differences in host colonisation and growth, including reduced hyphal branching and reduced detrimental effects on the host. In addition, glycogen-like deposits, which were abundant in the wild-type hyphae, were not evident in the mutants.

Unexpectedly, both the *thi1* mutant and wild-type strains formed some stromata on vegetative tissue. Electron microscopic examination revealed that the cells of epiphytic

hyphae found on the vegetative tillers typically were enlarged, lacking in cytoplasm and highly vacuolated, an ultrastructure similar to that found for hyphae growing in reproductive tillers. The mutants retained the ability to form conidia on the outer layer of the stomata. Extensive vascular colonisation and hyphal ramification in the mesophyll were common characteristics of stomata bearing regions. Although the morphology and ultrastructure of stomata formed on vegetative tillers is very similar to those on reproductive tillers, one significant difference was the presence of abundant glycogen-like deposits in hyphae of vegetative tillers. Furthermore, there were dramatic differences in the levels of glycogen-like deposits in hyphae in different regions of the vegetative tillers, indicating that the energy demand changes during stroma development. This is the first report of *E. typhina* forming stomata on non-inflorescence tillers.

Acknowledgements

I would first like to express my sincerest gratitude to my supervisor, Professor Barry Scott, who has given me the opportunity to work on this project and provided me with excellent guidance, support and inspiration throughout my study. I have improved so much from learning from you about science, about language and writing, and many others. I am also extremely grateful to my co-supervisor, scientist Mike Christensen at AgResearch Grasslands, for his encouragement, guidance in my study. I can not make such a progress in English without you. My gratefulness is also to my co-supervisor Dr Al Rowland for all the helps during the period of this study. Thank you, my supervisors. I hope I can continue learning from you in the future.

Throughout this study, I have also received lots of help and kindness from many other people. In regard to this I would like to express my thanks to Dr Rosie Bradshaw, Dr Max Scott, and Dr Jan Schmid in IMBS, Dr Brian Tapper in AgResearch Grasslands, and Dr Taha Al-Samarrai in HortResearch for suggestions and discussions which are valuable for my study. Thanks also to Dr Rissa Ota in IMBS for help in statistical analysis, to Raymond Bennett in HortResearch for microscopic technical work, to Kim Richardson in AgResearch Grasslands for clonal plants, to Wayne Simpson, Anouck de Bonth in AgResearch Grasslands for immuno blotting, and to Elizabeth Davies for HPLC analysis. My thanks also goes to Elizabeth Nickless, Ningxin Zhang, Jiancheng Song, Xuelei Li, Elizabeth Jaya, XingZhang Tong, Hongpin Jin in IMBS, Liyuan Chen, Shalom Basset in AgResearch Grasslands and Jingquan Feng, Qianhe Liu in Massey for technical advice and all sorts of help. Yanli, xiangqiang and Isaac, thank you for your friendships.

In particular, I would like express my gratitude to peoples in the Scott Base past and present, Carolyn, Michelle, Christina, Austen, Andrea, Brendan, Shuguang, Aiko, Kim, Simon, Sanjay, Hekei, Lisa, Emily, Renae, Raj, Rohan, Jonathan and Glenda thank you

for your technical advice, computing assistance, discussion and also proof-reading. Without your help, I would not achieved this progress. Brendan, thank you for you discussion on the nitrogen and carbon regulation. Glenda, thank you for your help in editing. Andrea, thank you for your proof-reading. Carolyn, Michelle, Christina and Austen, I feel warm and grateful to you whenever I think of you. Your warm friendship and lots of help made my life and study enjoyable in the lab. Michelle, you are not just my classmate. You are an angel in my life.

Finally, I would like to thank my parents, Boxun Zhang, Chanlian Huang; my husband Chunhong Chen and my daughter Jingyuan Chen, my brothers and sister-in-laws Shaohuai Zhang, Yuan Xie, Shize Zhang, and Piaopiao Long, my mother-in-law Shengyin Mi, and my niece Jingyi Zhang. Thank you for your love, encouragement and helps which always bring me strength and happiness. Jingyuan and Jingyi, your smile always bring me happiness and hope. Father, I am so sorry for your physically leaving us. I am proud of you and would like to live as you wish. Thank you for your unconditional love. I know I am lucky to have you as my dad.

God, my lord, thank you for your sending all these people in the world around me. The love, kindness, patience, sympathy and support form people are the true treasures in my life and in the world, and all these are from the bless of you. Thank you for your giving us life, love, hope and faith. I can not do these without you, my lord. May you be always with us, guide me, shape me, strengthen me and support me. I am desperate for you. Amen.

Thanks

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