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

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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 stromata. Extensive vascular colonisation and hyphal ramification in the mesophyll were common characteristics of stromata bearing regions. Although the morphology and ultrastructure of stromata 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 stromata on non-inflorescence tillers.

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Table of contents

List of figures

List of tables