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**CHARACTERIZATION OF TWO GENES INVOLVED IN  
*NEOTYPHODIUM LOLII* GROWTH.**

A thesis in partial fulfillment of the requirements for the degree of Master of Science in  
Biochemistry at Massey University, Palmerston North, New Zealand

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## ABSTRACT

*Neotyphodium lolii* is a filamentous fungus that forms symbiotic associations with *Lolium perenne*, growing in its intercellular spaces. It is a feature of the symbiosis that growth of the fungus and the plant is synchronized. When the grass leaf-blade grows, the fungus grows at the same rate, hence when the blade ceases extension the hyphae do likewise. In addition, *in planta* there is little hyphal branching, where as in culture hyphae branch at regular intervals. This suggests the existence of a regulatory mechanism *in planta* that partially dictates hyphal morphology and growth.

The criteria for choosing possible candidate genes relied on whether the gene had a function relating to hyphal branching and/or regulation of hyphal extension in several organisms. Three candidate genes were selected. Protein elongation factor 2 (*EF-2*; an elongation factor associated with the ribosome) was targeted to add more direct evidence to the high metabolic rate observed *in planta* using the GUS reporter gene by Tan *et al* (2001). Cell division control protein 12 (*CDC-12*); a septin which is involved in the construction of the 10 nm ring structure associated with cell division and whose mutation is lethal in yeast was chosen to help distinguish the growth mode of *N. lolii in planta*. A Stretch-activated Calcium Channel (*SACC*) which allows exogenous calcium into the cell upon application of lateral pressure on the membrane was targeted to help distinguish the possible recognition signal the hyphae make to elucidate when the host tissue is growing.

This project was then divided into four parts, one part per gene and a final part looking at the *in vitro* and *in vivo* expression of these genes. For the first three parts degenerate PCR was performed and appropriate-sized fragments cloned, sequenced and restriction mapped for *EF-2* and *CDC-12* (2066 bp and 514 bp respectively). Database searches were used to identify the sequences as potentially being the target genes. Degenerate PCR was unsuccessful for the *SACC*.

Southern blots were used to identify restriction enzymes for Inverse PCR; and this was used to obtain the remaining 5' and 3' regions of each target gene. Gene prediction software was used to predict gene structure; 5' and 3' RACE to confirm the length, introns and start/stop points of *EF-2* and *CDC-12* full gene transcripts (2,900 and 1,612 bp respectively). Internet-based sequence analysis tools subsequently were used to identify sequence features.

For the second part, expression of *EF-2* and *CDC-12* are investigated during various states of hyphal growth. Growth curves were constructed and *in vitro* expression analysis was achieved by Northern blot. The expression patterns of *EF-2* and *CDC-12* followed the growth state of *N. lolii*. RT PCR was used to confirm *in planta* expression of both genes and validate their uses for future studies.

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## ABBREVIATIONS

ATP	Adenosine 5'-triphosphate	h	Hour
GTP	Guanine 5'-triphosphate	mm	Millimeter
dNTP	Nucleotide 5'-triphosphate	cm	Centimeter
CDC	Cell cycle division protein	U	Units of Enzyme
EF	Protein Elongation factor		
SACC	Stretch-activated calcium channel		
mRNA	Messenger RNA		
MCS	Multiple cloning site		
GUS	$\beta$ -glucuronidase		
Gd <sup>3+</sup>	Galadeninum ion		
GST	Glutathione S-transferase		
kb	Nucleotides (Kilobases)		
b	Nucleotide (base)		
MOPs	3-N-Morpholinepropanesulfonic acid		
°C	Degrees centigrade		
$\mu$ L	Micro-liter		
mL	Milli-liter		
L	Liter		
pM	Pico-mole		
$\mu$ M	Micro-mole		
mM	Milli-mole		
M	Mole		
ng	Nanogram		
$\mu$ g	Microgram		
mg	Milligram		
g	Gram		
s	Second		