Investigation of the molecular basis of symbiosis between *Epichloë festucae* and perennial ryegrass

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Abstract

The symbiosis between the endophytic filamentous fungus *Epichloë festucae* and its plant host, perennial ryegrass (*Lolium perenne*), is a highly regulated mutualistic interaction which represents a good model system for the investigation of plant-fungal mutualism. Fungal signalling pathways play a crucial role in regulation of this interaction. While genes involved in the production of reactive oxygen species (ROS), as well as a member of the MAP kinase signalling pathway, have been shown to regulate maintenance of the mutualistic interaction, the signalling pathways responsible for regulation of this symbiosis are still relatively poorly understood.

In pathogenic fungi, members of calcium signalling pathways, such as Ca^{2+}/calmodulin-regulated kinases (CaMKs) and phosphatase (calcineurin), are required for normal host-pathogen interactions. Three genes encoding multifunctional CaMKs, *cmkA*, *cmkB* and *cmkC*, were identified in *E. festucae*, as well as one gene encoding the catalytic subunit of calcineurin, *cnaA*. Targeted replacements of these genes have identified a novel role for the fungal *cmkB* in the regulation of ion homeostasis and an important role for calcineurin for both culture growth and symbiosis maintenance. However, unlike the pathogenic fungi, *E. festucae* CaMKs do not appear to have a role in the regulation of the mutualistic interaction.

In order to identify new genes regulating the symbiosis, T-DNA mutagenesis was used to generate symbiotically defective *E. festucae* mutants. Two mutants, Ag51 and Ag212, with both in culture and *in planta* phenotypes, were identified. A detailed molecular analysis showed that Ag51 had a complex T-DNA insertion while Ag212 had a deletion of ten genes. Ag212 failed to establish plant infection and complementation experiments using cosmids identified candidate genes for both the in culture and *in planta* phenotype. Analysis of the colonization process showed that this mutant is defective in establishing a specific interaction between hyphal and plant cell walls, essential for the plant colonization.

This work provides new insights into calcium signalling in fungi and increases our understanding of plant-fungal mutualism.

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Abbreviations

A	Adenine
Amp^R	Ampicillin resistant
AMT	Agrobacterium mediated transformation
ATP	Adenine triphosphate
BLAST	Basic local alignment search tool
BLASTN	Nucleotide database search using a nucleotide query
BLASTP	Protein database search using a protein query
BLASTX	Protein database search using a translated nucleotide query
bp	Base pair(s)
BSA	Bovine serum albumin
Ca^{2+}/CaM	Ca^{2+}/calmodulin
CaMK	Ca^{2+}/calmodulin-dependent kinase, protein
CaMKK	Ca^{2+}/calmodulin-dependent kinase kinase
cmk	Ca^{2+}/calmodulin-dependent kinase, gene
cDNA	Complementary DNA
CDS	Coding sequence
CIAP	Calf intestinal alkaline phosphatase
DAB	3-3'Diaminobenzidine
DAG	Diacylglycerol
DIC	Differential interference contrast
DMSO	Dimethyl sulfoxide
dNTP	Deoxynucleotide triphosphate
EC	Ectopic
EGFP	Enhanced GFP
EGTA	Ethylene glycol tetraacetic acid
FGI	Fungal Genome Initiative
Gen^R	Geneticin resistant
GFP	Green fluorescent protein
GPCR	G protein-coupled receptor
HR	Hypersensitive response
Hyg^R	Hygromycin resistant

IM	Induction medium
ip	inoculation point
IP3	Inositol trisphosphate
Kan^R	Kanamycin resistant
Kb	Kilobases
KO	Knock-out
LB	Luria-Bertaini medium
LB	T-DNA left border
MAPK	Mitogen activated protein kinase
Mb	Megabases
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NBT	Nitroblue tetrazolium
NCBI	National centre for biotechnology information
Nox	NADPH oxidase
PCR	Polymerase chain reaction
PD	Potato dextrose
Phl^R	Phleomycin resistant
PKC	Protein kinase C
PLC	Phospholipase C
RB	T-DNA right border
REMI	Restriction enzyme mediated integration
RG	Regeneration medium
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room temperature
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase-polymerase chain reaction
SAM	Shoot apical meristem
TAIL-PCR	Thermal asymmetric interlaced-polymerase chain reaction
TBLASTN	Translated nucleotide database search using a protein query
T-DNA	Transfer DNA
TEM	Transmission electron microscopy

tRNA	Transfer RNA
WA	Water agar
WT	Wild-type
w/v	Weight/volume ratio