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Investigation into the role of PacC in
Epichloë festucae development and symbiosis
with perennial ryegrass

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Abstract

In order to survive and adapt to the environment, it is imperative for fungi to be able to sense and respond to changes in extracellular pH conditions. In ascomycetes, sensing of extracellular pH is mediated by the Pal pathway which is activated by alkaline pH. The signal is subsequently relayed to changes in gene expression by activation of the transcription factor PacC, which is known to regulate various fungal metabolic pathways; including ion tolerance, cell-wall integrity and secondary metabolism. The role of PacC in regulating fungal virulence and pathogenicity has also been studied in several pathogenic fungi, but to date not in a symbiotic fungus. *Epichloë festucae* is a biotrophic fungal endophyte that forms a stable symbiosis with the perennial ryegrass *Lolium perenne*. In this mutualistic interaction, secondary metabolites are produced by the fungus that confer bioprotection for the host, and the host in turn provides nutrients and a means of dissemination for the fungus.

In this study, deletion ($\Delta pacC$; acid-mimicking) and constitutively active ($pacC^{CA}$; alkaline-mimicking) mutants were generated to study the cellular roles of PacC in *E. festucae*. Deletion of *pacC* resulted in increased sensitivity to salt-stress and reduction in aerial hyphae formation, but did not affect the ability of the mutant to grow under alkaline pH conditions. The $pacC^{CA}$ mutant on the other hand showed greater sensitivity to cell-wall and temperature stresses, and was able to grow under both acidic and alkaline conditions. Aberrant intrahyphal hyphae formation and abnormal conidiation were additionally observed in the $pacC^{CA}$ mutant in culture, but hyphal fusion was unaffected. The expression of secondary metabolite genes both in culture and *in planta* was largely unaffected in both $\Delta pacC$ and $pacC^{CA}$ mutants, and expression analysis of genes known to be pH- and PacC-dependent in other fungi showed that these genes were generally pH- and PacC-independent in *E. festucae*. The deletion and constitutive active *pacC* mutants were still able to sense and modify extracellular pH. Deletion of *pacC* did not affect the endophyte-host interaction, but ryegrass plants infected with the $pacC^{CA}$ mutant were mildly hypertillered. Further examination of the growth of the $pacC^{CA}$ mutant in the plant revealed formation of aberrant convoluted hyphal structures and an increase in hyphal breakage; possible reasons for the observed host plant phenotype.

This study provides novel insights into the role of PacC in the agriculturally important endophyte, *E. festucae*. In this fungus, PacC regulates salt tolerance and cell-wall integrity, but not secondary metabolism and growth at non-neutral pH conditions. The results also show that PacC is involved in the symbiotic interaction between *E. festucae* and perennial ryegrass but is not crucial in the establishment and maintenance of the symbiosis.

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List of Abbreviations

aM	attomolar
Amp	ampicillin
Amp ^R	ampicillin-resistant
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BLAST	basic local alignment search tool
bp	base pair
cDNA	complementary DNA
cm	centimetre
Cp	crossing point
d	day(s)
DIC	differential interference contrast
DIG	digoxigenin
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dUTP	deoxyuridine triphosphate
EDTA	ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescent protein
ESCRT	endosomal sorting complex required for transport
Fab	fragment antigen-binding
g	gram
Gen	geneticin
Gen ^R	geneticin-resistant
GFP	green fluorescent protein
h	hour
Hyg	hygromycin
Hyg ^R	hygromycin-resistant
Kan	kanamycin
Kan ^R	kanamycin-resistant
kb	kilobase
kV	kilovolts
L	litre
LB	Luria-Bertani
LiAc	lithium acetate
M	Molar
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
mg	milligram
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
mRNA	messenger RNA
N.D.	not detectable
NADPH	nicotinamide adenine dinucleotide phosphate
NaOAc	sodium acetate
NB-LRR	nucleotide-binding leucine-rich repeat
NBT	nitro-blue tetrazolium
NES	nuclear export signal

ng	nanogram
NLS	nuclear localisation signal
nm	nanometre
nM	nanomolar
Nox	NADPH oxidase
PCR	polymerase chain reaction
PD	potato dextrose
PDA	potato dextrose agar
PEG	polyethylene glycol
PIPES	piperazine-N,N'-bis(2-ethanesulfonic acid)
pM	picomolar
PR	pathogenicity response
qRT-PCR	quantitative reverse transcription PCR
rcf	relative centrifugal force
RG	regeneration
RNA	ribonucleic acid
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	revolutions per minute
RT-PCR	reverse transcription PCR
s	second
SAM	shoot apical meristem
SDS	sodium dodecyl sulphate
SD-Ura	synthetic defined uracil dropout
SM	secondary metabolite
SOB	super optimal broth
spp.	species
SSC	saline-sodium citrate
TB	transformation buffer
TBE	Tris/Borate/EDTA
tBLASTn	search of translated nucleotide databases with a protein query
TMD	trans-membrane domain
ura	uracil
UTR	untranslated region
UV	ultraviolet
V	volt
v/v	volume to volume
vol	volume
w/v	weight to volume
WGA	wheat germ agglutinin
wpi	weeks post-inoculation
WT	wild-type
YPD	yeast-extract peptone dextrose
YRC	yeast recombinational cloning
μF	microfarad
μg	microgram
μL	microlitre
μm	micrometre
μM	micromolar