Adhesins – Do they play a role in the *Epichloë festucae* association with perennial rye grass?

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Abstract

Adhesins have been extensively studied and characterised in prokaryotes and yeast. It has been shown that these proteins are important in development, symbiosis and pathogenicity. However, less is known about the role adhesins play in filamentous fungi. Adhesin genes have been identified and functionally characterised in *Metarhizium robertsii* and recently studied in *Beauveria bassiana.* The insect pathogen *M. robertsii* has two adhesin genes, *Mad1* and *Mad2*, which were shown to be important in insect adherence or plant adherence respectively. *Epichloë festucae* has two adhesins, *adsA* and *adsB*, homologous to *Mad1* and *Mad2* respectively. Bioinfomatic analysis of *E. festucae* adhesins showed that in Fl1 *adsA* and *adsB* are separated by 25 genes. Analysis of *adsB* illustrated that the second adhesin gene is restricted to the Hypocreomycetidae. Phylogenetic analysis confirmed that *adsB* and *adsA* group separately in filamentous fungi.

In this study, deletion mutants of *E. festucae adsA* and *adsB* were used to determine if one or both adhesins played a role in establishment of the hyphal network in culture and in symbiotic association with *L. perenne*. Deletion of *adsA* did not alter the growth of the hyphae in culture or *in planta*. Although, the growth of the *adsB* mutant in culture was not affected the growth *in planta* was different to that seen in wild-type associations. Mild whole plant stunting and colonisation of the large vascular bundles indicates that *adsB* plays a role in the early development of the symbiotic association. In contrast to *M. robertsii* where only *Mad2* confers adherence of yeast to onion epidermal tissue, both *E. festucae adsA* and *adsB* confer adherence. The attachment of yeast cells expressing *adsA,* suggests that *adsA* does play a role in the symbiotic association. Physical attachment of the hyphae to host cells was not abolished when either *adsA* or *adsB* were deleted in *E. festucae,* suggesting gene redundancy in regards to physical attachment to the host tissue. This work provides insight into the role adhesins play in the symbiotic association of *E. festucae* and *L. perenne*.

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List of abbreviations

aa	Amino acid
Amp	Ampicillin
Amp^R	Ampicillin resistance
An	*Aspergillus niger*
bp	Base pairs
BLAST	Basic local alignment search tool
Bb	*Beauveria bassiana*
CFW	Calcofluor white
C	Plant cuticle
Cm	*Cordyceps militaris*
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic triphosphate
DIC	Differential interference contrast
DMSO	Dimethyl sulfoxide
ECS	Extracellular space
EGFP	Enhanced green fluorescent protein
EDTA	Ethlyene diamine tetra-acetic acid
FS	Flanking sequence
FGI	Fungal genome initiative
Fg	*Fusarium gramineaurm*
Fo	*Fusarium oxysporium*
Gen	Geneticin
Gen^R	Geneticin resistance
gDNA	Genomic DNA
GPI	Glycosylphosphatidylinositol
g	Gram
h	Hours
H	Hyphae

hph	Hygromycin phosphotransferase
Hyg^R	Hygromycin Resistance
ICS	Intercellular space
INVSc1	Invitrogen non-adherent variant *Saccharomyces cerevisiae*
kb	Kilobase
kV	Kilovolts
KO	Knock-out
Leu	Leucine
L	Litre
LB	Luria-Bertani
Mo	*Magnaportha oryzae*
mRNA	Messenger ribonucleic acid
Mr	*Metarhizium robertsii*
µg	Microgram
µL	Microlitre
µM	Micromolar
mg	Milligram
mL	Millilitre
mM	Millimolar
min	Minute
M	Molar
Nox	NADPH oxidase
ng	Nanogram
nm	Nanometre
NCBI	National Centre for Biotechnology
Nh	*Nectria haematococca*
BLASTn	Nucleotide database search using a nucleotide query
Nc	*Neurospora crassa*
NADH	Nicotinamide adenine dinucleotise
NADPH	Nicotinamide adenine dinucleotise phosphate
NA	Numerical aperture
ORF	Open reading frame

P	Phloem
Pa	*Podospora anserina*
PC	Plant cell
PEG	Polyethylene glycol
PCR	Polymerase chain reaction
PD	Potato dextrose
PDA	Potato dextrose agar
PDS	Post diauxic shift element
polyA	Polyadenylation site
pro	C6-Zn transcription factor
BLASTp	Protein database search using a protein query
RG	Regeneration
rpm	Revolutions per minute
RNA	Ribonucleic acid
S	Sclerenchyma
s	Seconds
Sc	*Saccharomyces cerevisiae*
sak	Stress activated kinase
SD-Ura-Leu	Synthetic define uracil and leucine dropout
SD-Ura	Synthetic defined uracil dropout
SP	Signal peptide
spp.	Species
STRE	Stress responsive element
TSS	Transcription start site
TEM	Transmission electron microscopy
Tr	*Trichoderma ressi*
Tv	*Trichoderma virens*
TE	Tris – EDTA
TBE	Tris/Borate/EDTA
UV	Ultraviolet
U	Unit
Ura	Uracil

Va	*Verticillium alfalfae*
V	Volts
VB	Vascular bundle
v/v	Volume/volume ratio
w/v	Weight/volume ratio
WGA	Wheat germ agglutinin
WT	Wild-type
X	Xylem
YE	Yeast extract
YRC	Yeast recombinational cloning
YPD	Yeast-extract peptone dextrose