Insect bioactive capabilities of *Epichloë festucae var lolii* AR48 infected *Lolium perenne*

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ABSTRACT

As the modern world expands and develops, new innovative methodologies for more efficient and environmentally friendly agricultural practices are required. Loss of crops through abiotic (*e.g.* drought) and biotic (*e.g.* herbivory) stresses has a major effect on the success of an agricultural industry. For animal production pasture crops are a key aspect of animal husbandry and directly affects yield and health. Symbiotic fungi belonging to the genus *Epichloë* form associations with cool season forage grasses and have been exploited as a new innovative method for insect pest management. Ryegrass infected with the asexual *E. festucae* var *lolii* strain AR48 has insect bioactivity against both the stem boring fly (SBF-*Ceradontha australis*) and cutworm moth caterpillar (CC -*Agrotis ipsilion*). The bioactive/s targeting both insects is currently unknown. The aim of this thesis was to identify the gene/s and/or bioactive/s present in AR48 infected ryegrass that have bioactivity against the SBF and/or CC. Two approaches were taken; the known insect bioactive secondary metabolite pathways in *Epichloë* were investigated in AR48 through bioinformatics and mass spectrometry, and the gene 'makes caterpillars floppy' (*mcf*), encoding an insect toxin like protein, was investigated through reverse genetics and insect bioactivity trials.

A new indole diterpene compound (IDT) was identified in AR48 infected plant material and this compound was absent in other *Epichloë* strains that do not have SBF and CC bioactivity. The same *mcf* gene allele as that present in the *E. typhina mcf* model, previously identified as having CC bioactivity, is present and predicted to be functional in AR48. The other *Epichloë* strains also have *mcf* genes predicted to be functional, however the *mcf* allele is different to the bioactive *E. typhina mcf* model. Overall, this project was able to identify a new IDT compound with potential insect bioactivity as well as identify two *Epichloë mcf* gene alleles that potentially have differing insect bioactivities.

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ABBREVIATIONS

%	Percentage
°C	degrees Celsius
3-GGI	3-geranylgeranyl indole
A600	Absorbance at 600 λ
aa	Amino acid
ACP	Acyl carrier protein domain
ADN	Artificial diet no choice
Amp	Ampicillin
AmpR	Ampicillin resistance
ASW	Argentine stem weevil
AT	Acyltransferase domain
Bcl-2	B-cell lymphoma 2
BH3	Bcl-2 domain three
BLAST	Basic Local Alignment Search Tool
BLASTn	BLAST search against a nucleotide database with a nucleotide sequence
BLASTp	BLAST search against a protein database with a protein sequence
BLASTx	BLAST search against a protein database with a translated nucleotide sequence
Bp	base pairs
BS	Blocking solution
C	Condensation domain
CADN	Cutworm moth caterpillar artificial diet no choice
CC	Cutworm moth caterpillar
cDNA	Complementary DNA
CDTN	Cutworm moth caterpillar detached tiller no choice
CDYE	Czapek Dox yeast extract
CHEF	Contour-clamped homogeneous electric field electrophoresis
CM	Cutworm moth
cm2	centimetres squared
CNF	Cytotoxic necrotizing factors
CoA	Coenzyme A
COMP	Gene complementation
CPD	Cysteine protease domain
CPD 2	Cysteine protease domain two
CPD1	Cysteine protease domain one
CT	Common toxic
CWPC	Cutworm moth caterpillar whole plant choice
CY	Cyclisation domain
CZ	Cell division zone
DH	Dehydratase domains
DMAPP	Dimethylallyl pyrophosphate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease

dNTP	Deoxyribonucleotide triphosphate
DSIR	Department of Scientific and Industrial Research
DTN	Detached tiller no choice
E	Epimerization domain
EAS	Ergot alkaloid
EDTA	Ethylenediaminetetraacetic acid
EZ	Expansion zone
FAD	Flavin adenine dinucleotide
FASTA	Fast-all
Fit	Fluorescens Insect Toxin
FPP	Farnesyl pyrophosphate
g	Gram
g	Acceleration due to gravity
Gen	Geneticin
GenR	Geneticin resistance
GGPP	Geranylgeranyl pyrophosphate
HGT	Horizontal gene transfer
hph	Hygromycin resistance conferring gene
Hyg	Hygromycin
HygR	Hygromycin resistance
IDT	Indole-diterpene
IGP	Indole-3-glycerol phosphate
IPP	Isopentenyl diphosphate
kb	Kilo base pairs
KR	Ketoreductase domains
KS	Keto-synthase domain
L	Litre
LADN	Light brown apple moth caterpillar artificial diet no choice
LB	Lysogeny broth
LBAM	Light brown apple moth caterpillar
LCMS	Liquid-chromatography mass spectrometry
LOL	Loline locus/gene cluster
LTM	Lolitrem locus/gene cluster
M	Moles per litre
MIDAS	Modular Idempotent DNA Assembly System
m/s	Meters per second
Mcf	Makes caterpillars floppy
MEP	2-Methyl-D-erythritol-4-phosphate
mg	Milligram
mins	Minutes
MITEs	Miniature inverted transposable elements
mL	Millilitre
mM	millimoles per litre
mm	Millimetre
MQ	Milli-Q water
mRNA	Messenger RNA

MS	Mass spectrometry
MT	Methyltransferase domains
ng	Nanograms
NMR	Nuclear magnetic resonance
nptII	Geneticin resistance gene
NRPS	Non-ribosomal peptide synthetase
NZGL	New Zealand Genome Limited
OM	Osmoregulation buffer
OX	Oxidation domain
PADN	Porina caterpillar artificial diet no choice
Pax	Paxilline
PC	Porina caterpillar
PC1	Physical containment one
PC2	Physical containment two
PCR	Polymerase chain reaction
PD	Potato dextrose
PDTN	Porina caterpillar detached tiller no choice
PEG	Polyethylene glycol
PER	Peramine
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
PKS	Polyketide synthase
PM	Porina caterpillar
PM	Porina moth
RG	Regeneration
RNA	Ribonucleic acid
Rnase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT-qPCR	Real time quantitative PCR
RTX	Repeats-in-toxin
SADN	Stem boring fly artificial diet no choice
SBF	Stem boring fly
SD	Secretion domain
SDS	Sodium dodecyl sulfate
SDTN	Stem boring fly detached tiller no choice
sec	Seconds
SM	Secondary metabolite
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
ST	Sorbitol Tris
STC	Sorbitol Tris calcium chloride
SWPC	Stem boring fly whole plant choice
TB	Tris-boric
TBE	Tris-boric acid-EDTA
tBLASTn	BLAST search against a nucleotide database with a protein sequence
TE	Thioesterase domain

Tm	Melting temperature
TMD	Transmembrane domain
UV	Ultra violet
V	Volume
v/v	Volume/volume
w/v	Weight/volume
WA	Water agar
WPC	Whole plant choice
WT	Wild type
Δ	Gene deletion
µg	Microgram
µL	Microliter
µm	Micrometre