A metabolomic analysis of G-protein signalling mutants of *Stagonospora nodorum*

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“Up and at them”

Rainier Wolfcastle

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Acknowledgements

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Abstract

Stagonospora nodorum is the causal agent of Stagonospora nodorum blotch (SNB) of wheat. This fungus has cost the Australian grains industry upwards of 100 million dollars (AUD) p.a. in recent growing seasons, making it one of the most agriculturally damaging pathogens in Australia.

Disease severity is governed by the polycyclic lifecycle of *S. nodorum*, requiring a succession of spore inoculum arising from the asexual fruiting body of the fungus, known as the pycnidium. The resultant fungal density will determine the level of damage and ultimately influence the grain yield of the plant. G-protein signalling through the heterotrimeric G-protein is a biochemical mechanism used by *S. nodorum* in the host-pathogen interaction and has been linked to important biological processes including asexual sporulation. In this work, the unique phenotypes of three mutant strains of *S. nodorum*; each lacking either the Gα (Gna1), Gβ (Gba1), or Gγ (GgaA) subunit of the heterotrimeric G-protein were explored, and the biochemistry underpinning the phenotypes assessed by metabolomics.

The mutant strain *S. nodorum gga*A was created by homologous recombination of the *Gga*A gene for comparison with the previously created *gna*1 and *gba*1 strains. All strains possessed developmental defects and reduced pathogenicity on the wheat plant. Growth assays uncovered differences in carbon source utilisation between the strains. Asexual sporulation was monitored by light microscopy; with the differentiation of mutant mycelia into pycnidia found to occur only after a comparatively longer culture time than in wild type, and at a reduced temperature. Until this time, asexual sporulation is completely abolished in the mutant strains. The matured pycnidia also possessed an irregular morphology. These results identified an association of all three G-protein subunits in asexual sporulation in *S. nodorum*.

Metabolites were isolated from *S. nodorum* mycelia for gas chromatography-mass spectrometer (GC-MS) analysis. An assessment of existing metabolomic methods identified some key steps in the sample preparation employed prior to injection into the GC-MS. Quenching the fungal metabolism

upon harvesting, drying the fungal mycelia prior to metabolite extraction and isolation, and lyophilisation of the fungal metabolites in preparation for chemical derivatisation; each improved the metabolite recovery and overall reliability of the metabolomic analyses. These methods were applied to the metabolomic characterisations that followed.

Metabolite extracts from the *in vitro* cultured fungal strains were analysed using a single-quadrupole GC-MS and the recorded analytes cross-refereces to purchased metabolite standards for identification. Changes in the accumulation of various carbohydrates were apparent in the mutant metabolomes. Of those, the altered abundances of the metabolites glucose and trehalose are believed to in part explain or be consequential to the sporulation phenomena of these strains. Metabolomic analysis of the mutant strains in differentiating from a non-sporulating to a sporulating phenotype revealed the specific association of a number of metabolites with each of the two phenotypic classifications. Many of which have been targeted for identification in future studies. Among those identified was again trehalose, providing further evidence for it having a role in the asexual sporulation of this fungus.

These results have demonstrated the requirement for Gna1, Gba1 and GgaA in regulating developmental processes and the pathogenesis of *S. nodorum*, and added significantly to the biochemical dissection of asexual sporulation in this fungus.

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Gene/protein nomenclature

Genes are presented in italics, the first letter of which is in uppercase (eg. *Abc*1). Proteins are non-italicised, with the first letter in uppercase (eg. Abc1). Deleted genes are italicised and in lowercase (eg. *abc*1). Fungal mutant strains are identified by the deactivated gene name, italicised and in lowercase, as described for deleted genes.

Abbreviations and Units

3'	3 prime
5'	5 prime
ATP	adenosine triphosphate
aa	amino acid
AUD	Australian Dollars
ACNFP	Australian Centre for Necrotrophic Fungal Pathogens
bp	basepair
BLASTP	basic local (protein) alignment search tool
CE	capillary electrophoresis
cm	centimetre
CSF	cerebral spinal fluid
CS	complete supplement
CT	cycle threshold
cAMP	cyclic adenosine monophosphate
CzV8	Czapek Dox V8 juice
Dpi	days post inoculation
°C	degrees celcius
eV	electron volts
milli-Q	0.22µm Millipore membrane-filtered deionised water
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphate
DLA	detached leaf assay
L-DOPA	dihydroxyphenylalanine
dpi	days post inoculation
EI	electron ionization
ESI-MS/MS	electrospray ionisation tandem mass spectrometry
E.C	Enzyme Commission/accession number
fmol	femtomoles
FTMS	fourier transform MS
FT-ICR	fourier transform ion cyclotron resonance
GC	gas chromatographer
GC-MS	gas chromatography mass spectrometry

GC-(Q)MS	GC single quadrupole MS
gDNA	genomic DNA
Gα	G-protein alpha subunit
Gβ	G-protein beta subunit
Gβγ	G-protein beta-gamma subunit dimer
GPCR	G-protein coupled (plasma membrane) receptor
GFP	green fluorescent protein
Gγ	G-protein gamma subunit
g	gram
g	gravitational force
GTPase	GTP hydrolase
GDP	guanisine diphosphate
GTP	guanisine triphosphate
G-protein	guanisine-binding protein
Hz	Hertz
HSD	honestly significant difference
HST	host-specific toxin
G_s	inhibitory G-protein
P_i	inorganic phosphate
IT	ion trap
kb	kilobase pairs
LOD	limit of detection
MS	mass sectrometer
MST	mass spectral tag
m/z	mass-to-charge
MEOX	methoxylamine hydrochloride
m	metre
µg	microgram
µl	microlitre
µM	micromolar
mg	milligram
ml	millilitre
mm	millimetre
mm^2	millimetres squared
mM	Millimolar
MYA	million years ago
MM	minimal medium
M	molar
MW	molecular weight marker
NIST	National Institute of Standards and Technology
NE	necrotrophic effector
MSTFA	N-Methyl-trimethylsilyltrifluoroacetamide
NS	not significant
NTC	no-template control
NMR	nuclear magnetic resonance
nt	nucleotide

p.a	per annum
PPP	pentose phosphate pathway
PFTBA	perfluorotributylamine
PEG	polyethylene glycol
PCR	polymerase chain reaction
PDA	potato dextrose agar
PC	principal component
PCA	principal component analysis
PKA	protein kinase
Q	quadrupole
QTOF	quadrupole TOF
Res.	resolution
RT-qPCR	quantitative real-time PCR
RI	retention index
RT	retention time
rpm	revolutions per minute
SCH	short-chain dehydrogenase
SNB	Stagonospora nodorum blotch
SN15	*Stagonospora nodorum* strain 15 (wild-type)
Std	standard deviation
SNOG	*Stagonospora nodorum* gene
G_s	stimulatory G-protein
TBE	tris-borate EDTA buffer
TWA	tap water agar
MS/MS	tandem mass spectrometry
TOF	time of flight
TIC	total ion count/chromatogram
TCA	tricarboxylic acid
TMS	trimethylsilyl ($Si(CH_3)_3$)
QQQ	triple quadrupole
GC×*G*C	two dimensional gas chromatography
LC×LC	two dimensional liquid chromatography
U	Unit
UTR	untranslated region
V	volts
v/v	volume per volume
wk	week
w/v	weight per volume
WT	wild-type
YPD	yeast potato dextrose