Molecular Quest for Avirulence Factors in *Venturia inaequalis*

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Abstract

The molecular basis for the gene-for-gene relationship of V_m-resistance in apple to *Venturia inaequalis* was investigated. Incompatible reactions involved a hypersensitive response (HR), which was accompanied by the accumulation of dark brown pigments and autofluorescent materials in epidermal and mesophyll cells at the site of invasion. Cell-free culture filtrates of the avirulent isolate elicited an HR in the *Vm* host (h_5) leaves, but not in the susceptible host (h_1). The elicitor activity was resistant to boiling but was abolished by proteinase K digestion. Elicitation of HR was used to monitor purification of the avirulence factor, AVRVm, from liquid cultures of the avirulent isolate following ultrafiltration, acetone precipitation and ion-exchange chromatography. The purest fraction contained three major proteins all with low isoelectric points (pI 3.0-4.5). The fraction also elicited HR on the differential host h_4, but not on other resistant hosts (h_2, h_3 and h_6) tested. Three candidate AVRVm proteins were identified and amino acid sequences were obtained using Edman degradation and mass spectrometry. Nucleotide sequences corresponding to these proteins were found in databases of *V. inaequalis* expressed sequence tags. There were no polymorphisms evident between avirulent and virulent isolates (representing races 1 and 5 respectively) either at genomic DNA or cDNA level of the full open reading frames. RT-PCR revealed that all genes were expressed in both avirulent and virulent isolates during *in vitro* and *in planta* growth. All three genes showed similar levels of expression between avirulent and virulent isolates during their *in vitro* growth. However, preliminary RT-PCR experiments showed that two of these genes were likely to be expressed at lower levels in the virulent compared with the avirulent isolate during compatible infection. Implications of this difference in expression and the future experiments to identify the genuine *AvrVm* gene were discussed.

Dedication

To my mother, Mi Nyunt Kyi, who unconditionally supported (and she still does) me with love and everything else I asked of her throughout this thesis and my life.

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Abbreviations

SI units (Système International d'unités) and their derived units were used through out this thesis. One-letter symbols for amino acids and nucleotides were according to IUPAC-IUB (The International Union of Pure and Applied Chemistry -International Union of Biochemistry) guidelines (International Union of Biochemistry, 1978).

°C	degree Centigrade
2D	two-dimensional
APAF	Australian Proteome Analysis Facility
Avr	avirulence
AvrVx	avirulence gene from *V. inaequalis* corresponding to resistance gene V_x
AVRVx	avirulence protein from *V. inaequalis* corresponding to resistance Protein V_x
bp	base pair
CC	coiled-coil domain
cDNA	complementary deoxyribonucleic acid
CF	cell-free culture filtrate
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
cM	centi-Morgan
d	day
Da	Dalton
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E-value	Expect value
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionisation
EST	expressed sequence tag
h	hour
HPLC	high performance liquid chromatography
HR	hypersensitive response
h_x	*Malus* differential host, x = number
IEF	isoelectric focusing
IPG	immobilised pH gradient
LB	Luria broth
LC	liquid chromatography
LRR	leucine rich repeat
LZ	leucine zipper
MALDI-TOF	matrix-assisted laser desorption/ionisation-time of flight
MES	2-[N-morpholino]ethanesulphonic acid
min	minute

mRNA	messenger ribonucleic acid
MS	mass spectrometry
MW	molecular weight
MWCO	molecular weight cut off
N-terminal	amino terminal
NBS	nucleotide binding sites
No.	number
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDA	potato dextrose agar
PDB	potato dextrose broth
pI	isoelectric point
PK	protein kinase
PR	pathogenesis-related
PTH	3-phenyl-2-thiohydantoin
PVDF	polyvinylidene difluoride
Q-TOF	quadrupole-time of flight
R	resistance
RGH	resistance gene homologs
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-primed polymerase chain reaction
SDS	sodium dodecyl sulphate
TAE	tris-acetate EDTA running buffer
TBP	Tributylphosphine
TFA	trifluoroacetic acid
TIF	tagged image format
TIR	Toll/Interleukin 1 receptor
TM	transmembrane region
Tm	melting temperature
U	unit
UV	ultra-violet
v/v	volume per volume
Vh	Volt hours
V_x	Resistance gene against *V. inaequalis* from *Malus* spp., x = initial of the species of *Malus* (e. g., V_m = *V. inaequalis* resistance gene from *M. micromalus*)
w/v	weight per volume