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Characterisation of Mycoviruses in the Plant Pathogenic Fungus, *Botrytis cinerea*

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ABSTRACT

In recent years a lot of interest has focussed on the role of mycoviruses (fungal viruses) that confer reduced pathogenicity to their fungal hosts. In several plant pathogenic fungi viral double-stranded RNA (dsRNA) elements have been shown to be the agents responsible for this attenuation of virulence.

In New Zealand and other temperate climates the plant pathogenic fungus, *Botrytis cinerea* is an important pathogen of many horticultural crops. Control of this grey mould disease still relies heavily on the use of fungicides to augment cultural practices. Consequently, there is now increasing interest in the use of biological control as an alternative means of control.

This study reports the first large-scale survey of dsRNA elements in *B. cinerea.* Analysis of 200 New Zealand isolates from a range of hosts revealed 72% contained dsRNA, with high variation in dsRNA profiles, both within and between groups of fungal isolates from different hosts. DsRNA has been associated with isometric particles in many different fungi. In this study virus-like particles (VLPs) were observed in three of five dsRNA-containing isolates examined. Morphological types included isometric particles of varying size classes (approx. 30, 35, 40 and 45 nm) and bacilliform particles (approx. 25 x 63 nm). In five isolates apparently lacking dsRNA no isometric or bacilliform particles were found but flexuous rods with a modal length of approx. 720 nm were detected in one of these isolates. Total RNA extraction of the various partially purified VLP preparations gave similar profiles to those obtained from mycelial dsRNA extractions of the same isolates, suggesting that the VLPs comprise encapsidated dsRNA. Comparison of 12 dsRNA-containing and 12 dsRNA-free isolates indicated slight differences between the two groups in radial growth rate, virulence on bean leaves and sclerotial number, but the differences were minor. In summary, while dsRNA was found to be common among *B. cinerea* isolates in this study, the evidence did not reveal any marked phenotypic effects of dsRNA presence.

The putative flexuous rod-shaped virus particles in *B. cinerea* were studied further. Similar particles have been observed in fungi before but none have been characterised at

the molecular level. This study reports the first complete nucleotide sequences of two such mycoviruses, *Botrytis* Virus F (BVF) and *Botrytis* Virus X (BVX), and also the first molecular characterisation of viruses infecting *B. cinerea*. The mycoviruses, which were both present in the same *B. cinerea* isolate, were similar in size and morphology to ssRNA plant viruses of the family Potexviridae. BVF and BVX contained ssRNA genomes of 6827 and 6966 nucleotides respectively, excluding poly(A) tails. Computer analysis of the genomic cDNA sequence of BVF revealed three potential open reading frames (ORFs) encoding putative proteins of 20 kDa (ORF1), 212 kDa (ORF2) and 32 kDa (ORF3). ORF1, which overlapped ORF2 by 467 nt, did not reveal significant homology with known protein sequences. ORF2, which showed significant sequence identity to the replicase proteins of the plant virus families Tymoviridae and Potexviridae, contained a putative opal readthrough codon intermediate between the helicase and RNA-dependent RNA polymerase (RDRP) regions. ORF3 shared amino acid homology with coat proteins of the family Potexviridae. A putative defective RNA (D RNA) of 829 nt was also detected which consisted of a 5' region of the parental genome fused in-frame to the terminal 3' end, complete with poly(A) tail. BVX revealed five potential ORFs encoding putative proteins of 158 kDa (ORF1), 30 kDa (ORF2), 44 kDa (ORF3), 14 kDa (ORF4) and 14kDa (ORF5). ORF1 showed significant amino acid sequence identity to the replicase proteins and ORF3 to the coat proteins of the Potexviridae. The remaining ORFs did not reveal significant homology with known protein sequences.

This is the first report of sequence similarities between mycoviruses and the plant virus families Potexviridae and Tymoviridae. Comparisons of sequence and genome organisation suggest that the mycoviruses BVF and BVX may each belong to a new as yet unassigned genus in the plant virus family Potexviridae. An unexpected result from this study was the high level of identity observed between the derived amino acid sequences of the mycoviruses and plant viruses including a region of ~70% for the RNA-dependent RNA polymerase (RDRP) region of BVX and similar regions in several potex-like viruses of *Allium* spp. This high level of interviral homology has not previously been reported between the plant and fungal kingdoms and is possibly indicative of a recent divergence from a common ancestor. These results suggest that horizontal gene transfer between the plant and fungal kingdoms may occur more

frequently than has been appreciated to date. If so it may play a significant role in viral evolution.

In addition to providing novel phylogenetic information on the relationship between these morphological types of viruses in fungi and in plants, characterisation of these mycoviral genomes provides a possible strategy for incorporating genes into *Botrytis* using viruses as vectors. In this context *Botrytis* strains infected with modified viruses could provide a new approach for the biocontrol of grey mould.

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ABBREVIATIONS

(+)	positive strand
A	adenine
aa	amino acid(s)
B. cinerea	*Botrytis cinerea*
bp	base pair(s)
C	cytosine
cDNA	complementary DNA
CP	coat protein
C. parasitica	*Cryphonectria parasitica*
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DrBr	dicarboximide-resistant, benzimidazole-resistant
DrBs	dicarboximide-resistant, benzimidazole-sensitive
D RNA	defective RNA
DsBr	dicarboximide-sensitive, benzimidazole-resistant
DsBs	dicarboximide-sensitive, benzimidazole-sensitive
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
EDTA	ethylene diamine tetra-acetic acid
g	acceleration of gravity
G	guanine
GCG	Genetics Computer Group (University of Wisconsin)
h	hour(s)
kb	kilobase(s)

kbp	kilobase pair(s)
kDa, (K)	kilodalton(s)
MEA	Malt extract agar
min	minute(s)
mRNA	messenger RNA
MTR	methyltransferase
nm	nanometre(s)
nt	nucleotide(s)
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
RDRP	RNA-dependent RNA polymerase
RNA	ribonucleic acid
R. solani	*Rhizoctonia solani*
RT-PCR	reverse transcription-polymerase chain reaction
SDS	sodium dodecyl sulphate
SSC	saline-sodium citrate
T	thymine
TBE	tris-borate
Tris	tris (hydroxymethyl)-amino-methane
U	unit or uracil
v	volume
VC	vegetative compatibility
VLP	virus-like particle
w	weight