Development of Mycovirus Causing Hypovirulence to Phytopathogen as Biocontroller

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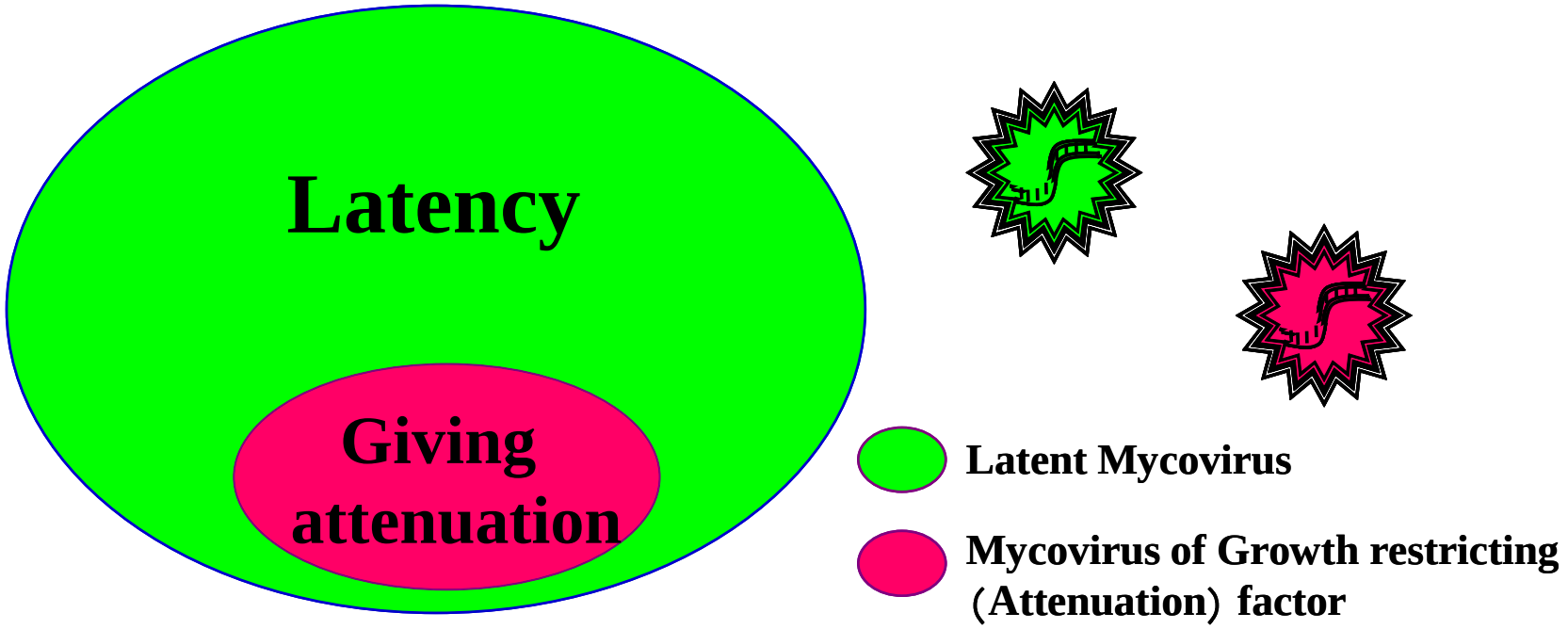
Fig.1 Mycovirus of fungi

“Fungi having genome of double-stranded RNAs are collectively called Mycovirus.”



- **Most of mycovirus do not influence host-fungi clearly.**

- **Some mycoviruses play a role in restricting growth of host fungi and attenuating pathogens.**

Mycoviruses associated with host virulence modulation

Spices	Family	Nucleic Acid	Host (disease)
Cryphonectria parasitica hypovirus	*Hypoviridae*	dsRNA (9-13kbp)	*C. parasitica* (Chestnut blight)
Helminthosporium victoriae virus 190s	*Totiviridae*	dsRNA (5kbp)	*H. victoriae* (Victoria blight of oats)
Helminthosporium victoriae virus 145s	*Chrysoviridae*	Four dsRNA (2.8-3.6kbp)	*H. victoriae* (Victoria blight of oats)
Unclassified virus	——	dsRNA (3.6kbp)	*Rhizoctonia solani* (Rhizoctonia disease of potato)
Ophiostoma novo-ulmi mitovirus	*Narnaviridae*	ssRNA (about 2.5kbp)	*O. novo-ulmi* (Dutch elm disease)

Mycoviruses related to host fungal virulence modulation were screened in several strains of ***Alternaria alternata*** **or** ***Magnaporthe oryzae.***

Target in middle term：Research and nature analysis of mycoviruses to restrict Magnaporthe oryzae and A.Alternate.

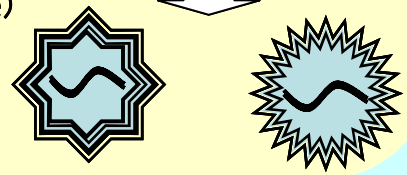
Diseased tissues on an oryza sativa leaf

Separation

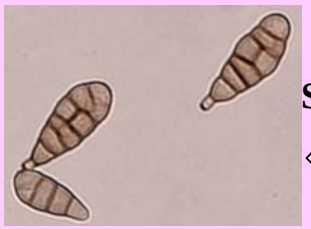
Magnaporthe oryzae

Poor growth of Hyphae
Isolation from stock cultures of fungi

Mycovirus
（Double-stranded RNA genome）



A.Alternate



Poor growth of Hyphae
Isolation from stock cultures of fungi

Seperation

Soil or fruits, vegetables

Mycovirus
（Double-stranded RNA genome）

Target in result

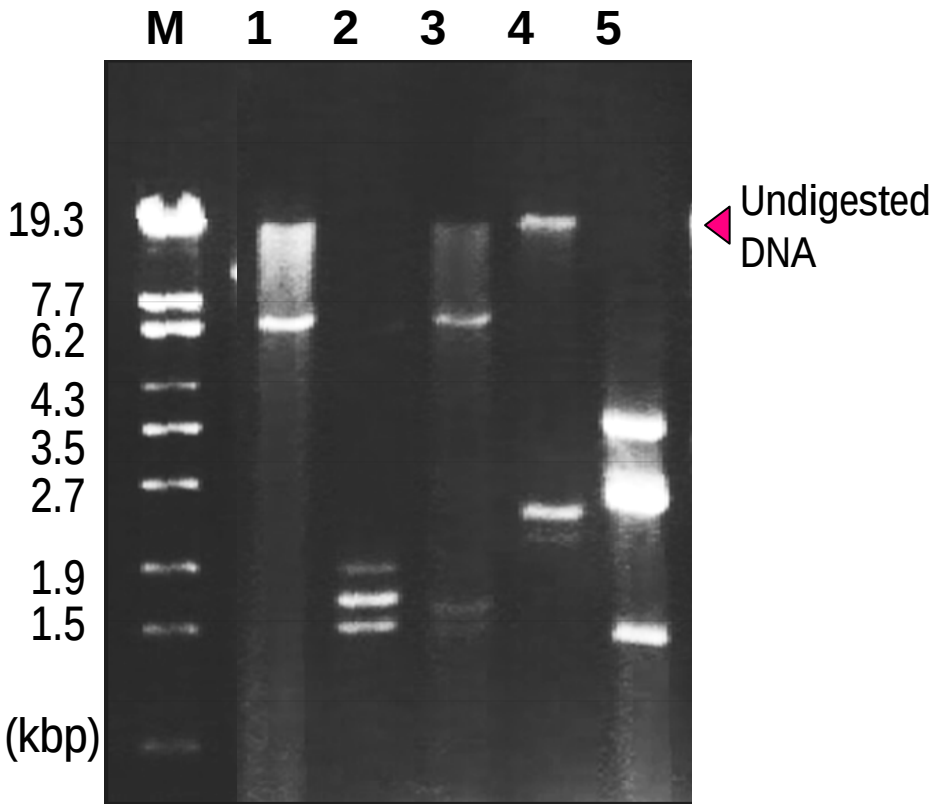
1. Identification of mycovirus to attenuate host pathogen
2. Identification of genetic structure and pysical chemical properties of the virus

Mycovirus containing four double-stranded RNAs affects host fungal growth in *Alternaria alternata.*

Detection of dsRNAs in *Alternaria alternata*

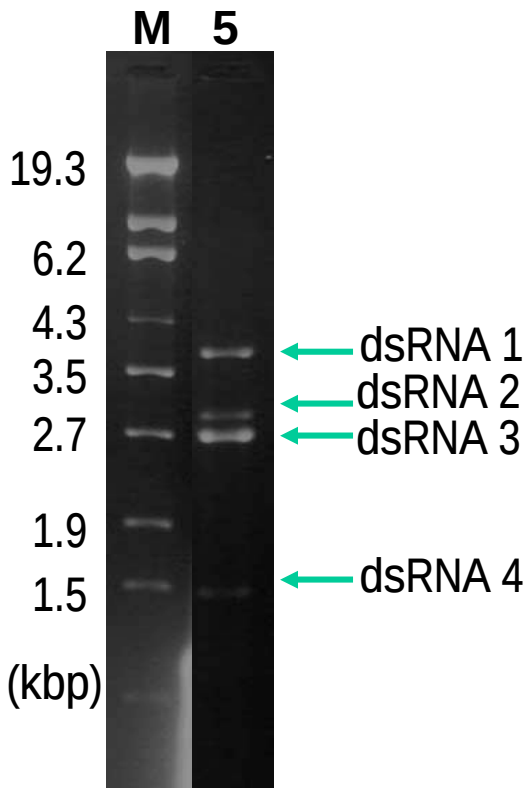
A

Total nucleic acids treated with DNase I &S1Nuclease



B

CF-11 column purified dsRNA



M. DNA marker
1. *A. alternata* (12L-5)
2. *A. alternata* (22L-5)
3. *A. alternata* (15F-3)
4. *A. alternata* (O-159)
5. *A. alternata* (EGS 35-193)

Various sized (from 1.5 to 6.2kbp) dsRNAs were detected in *A. alternata*.

Influence of dsRNAs on phenotype of *A. alternata*

① EGS 35-193;
original strain

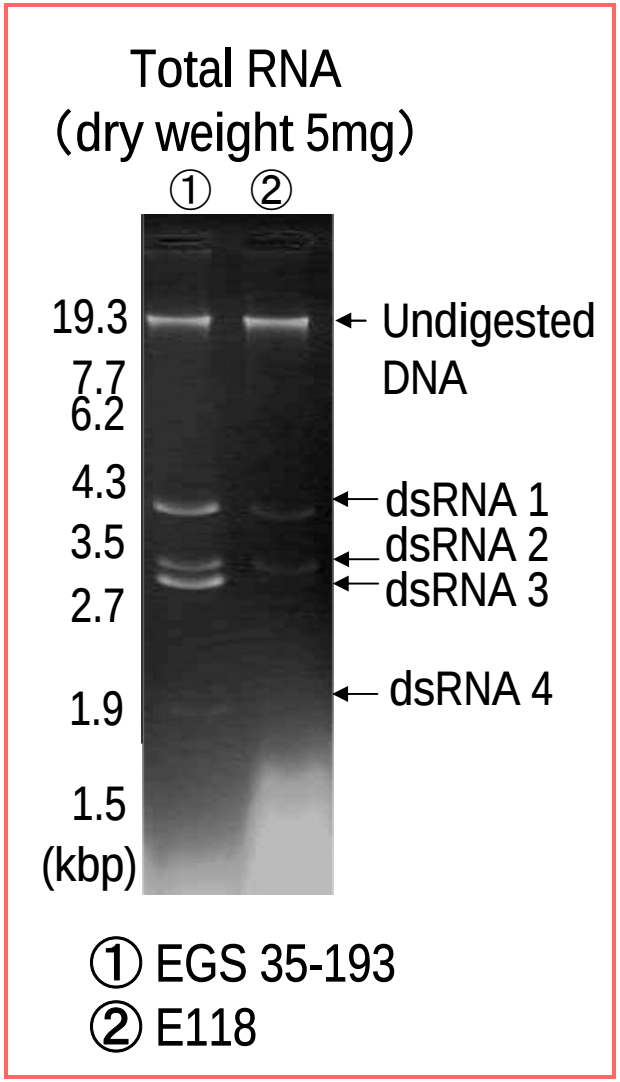
- late growth
- reduced number of aerial mycelia
- irregular pigmentation
- detection of four dsRNAs

Cure-treatment

Expose to 1 μ g/ml Cycrohexamaide
Hyphal tip cultivation (Repeated)

② E118;
dsRNA cured strain

- normal growth
- large number of aerial mycelia
- normal pigmentation
- lower concentration of dsRNAs

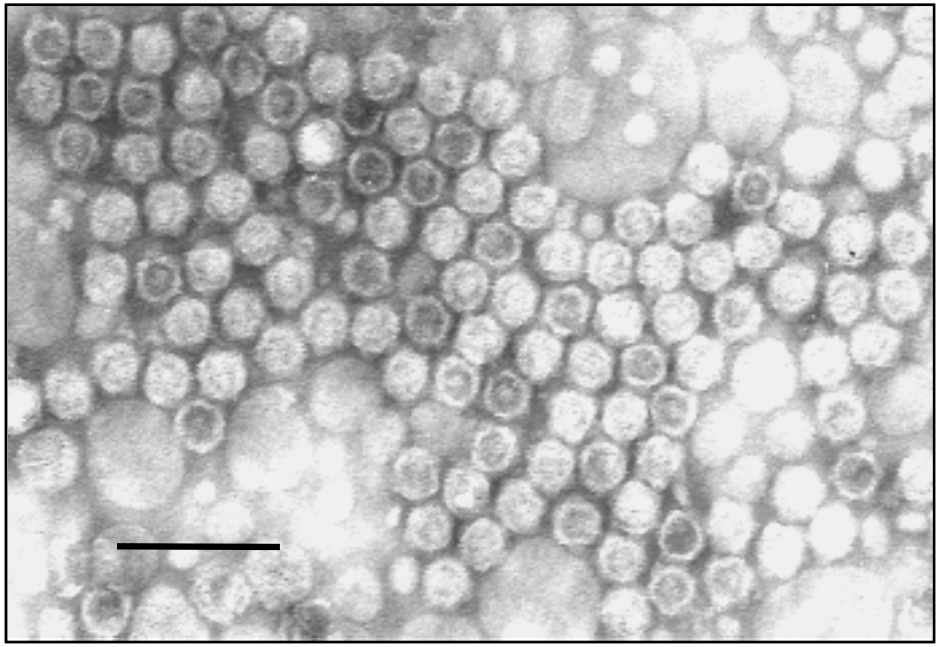


High copy number of viral dsRNAs caused phenotypic changes on their host, *A. alternata*.

Virus-like particles of *A. alternata* mycovirus

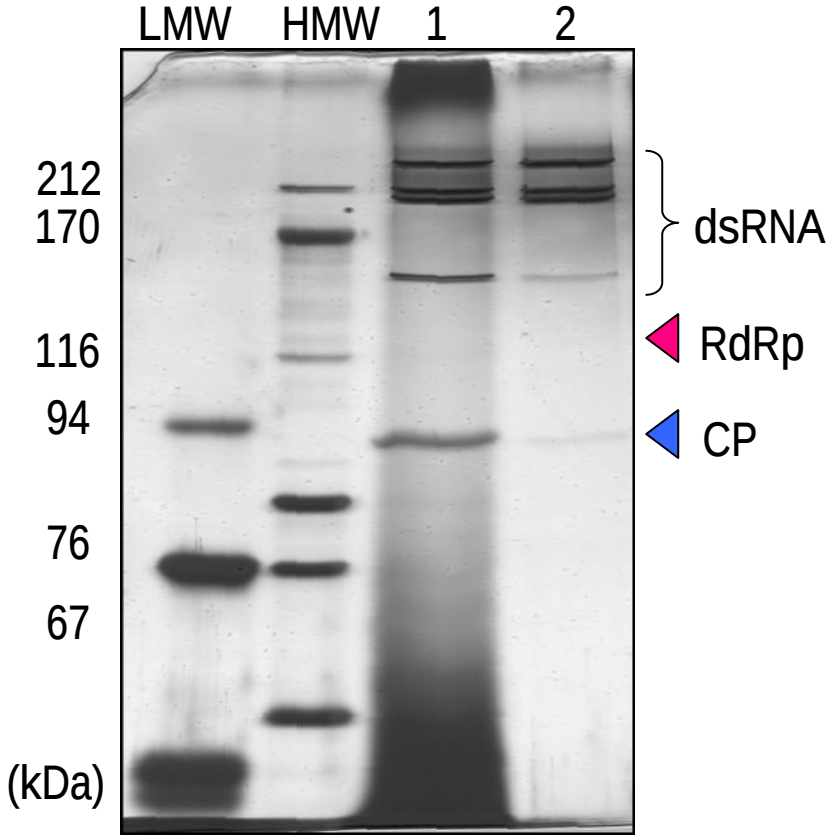
Electron micrograph of virus-like particles

Bar = 100 nm



Purified virus preparation was stained with 2 % uranyl acetate

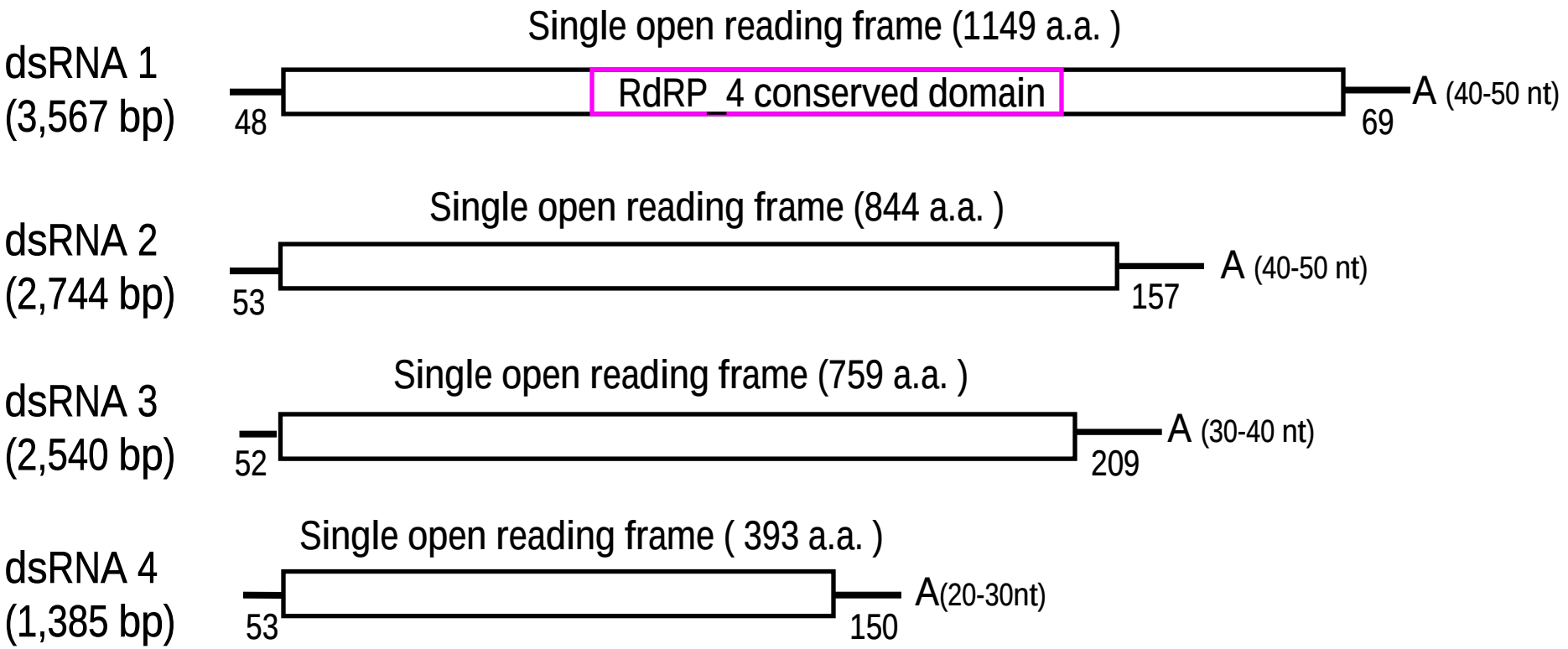
Silver stain of SDS-PAGE of virus preparation



1. Virus preparation
2. Purified virus

Isometric virus particles with a diameter of about 33 nm were observed.

Genome organization of *A. alternata mycovirus*

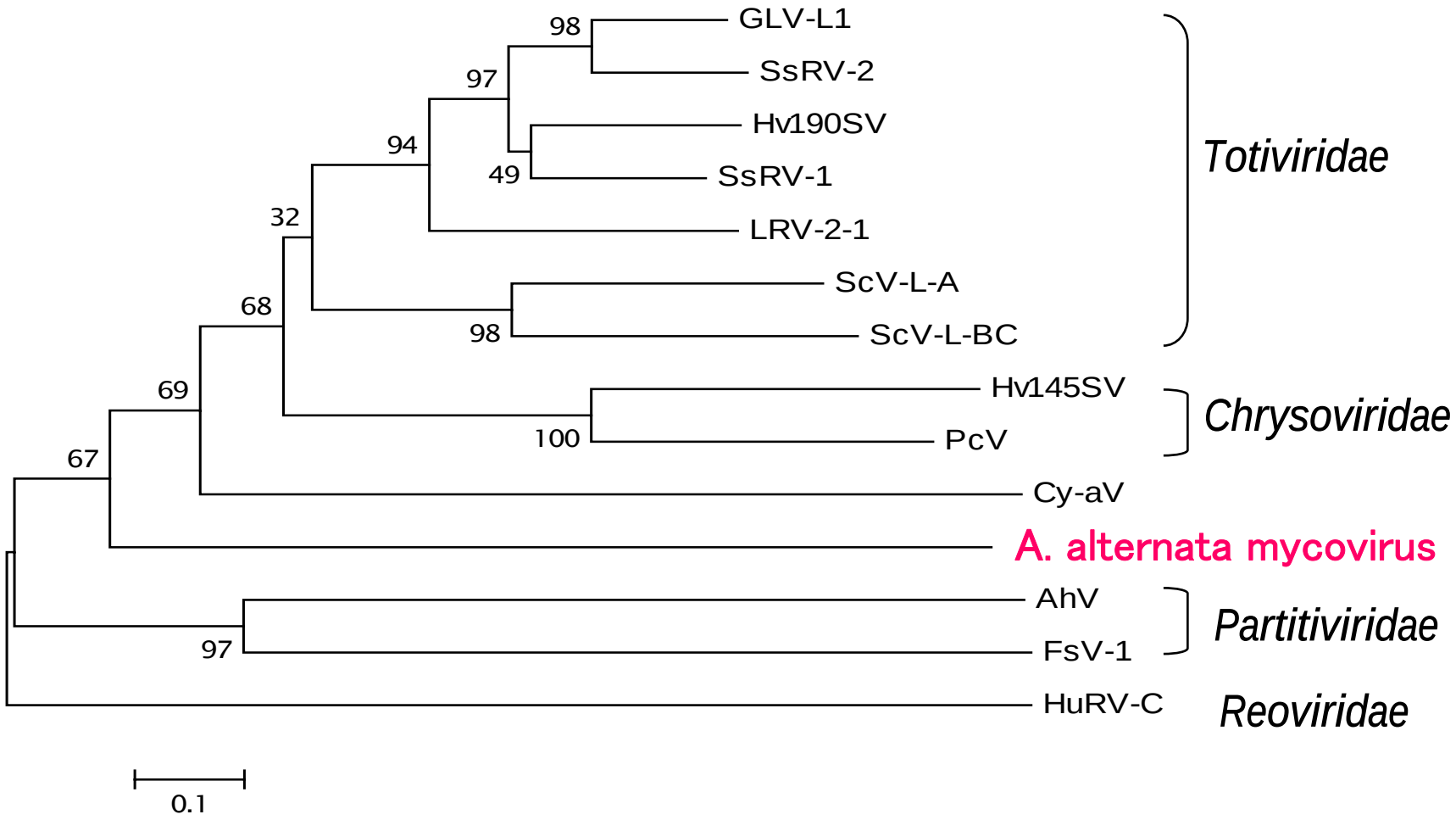


RdRP_4 : Viral RNA-directed RNA-polymerase

This family includes RNA-dependent RNA polymerase proteins (RdRPs) from Luteovirus, Totivirus and Rotavirus.

dsRNA 1 encodes a single open reading frame that contains a *Totivirus-like* RdRP (RdRP_4) conserved domain.

Phylogenetic tree of the RdRp regions of *A. alternata mycovirus* and selected mycoviruses



The *A.alternaria mycovirus* is related to *Crysoviridae*, *Partitiviridae*, *Totiviridae*.

Comparison between AaV and *mycovirus* in three families

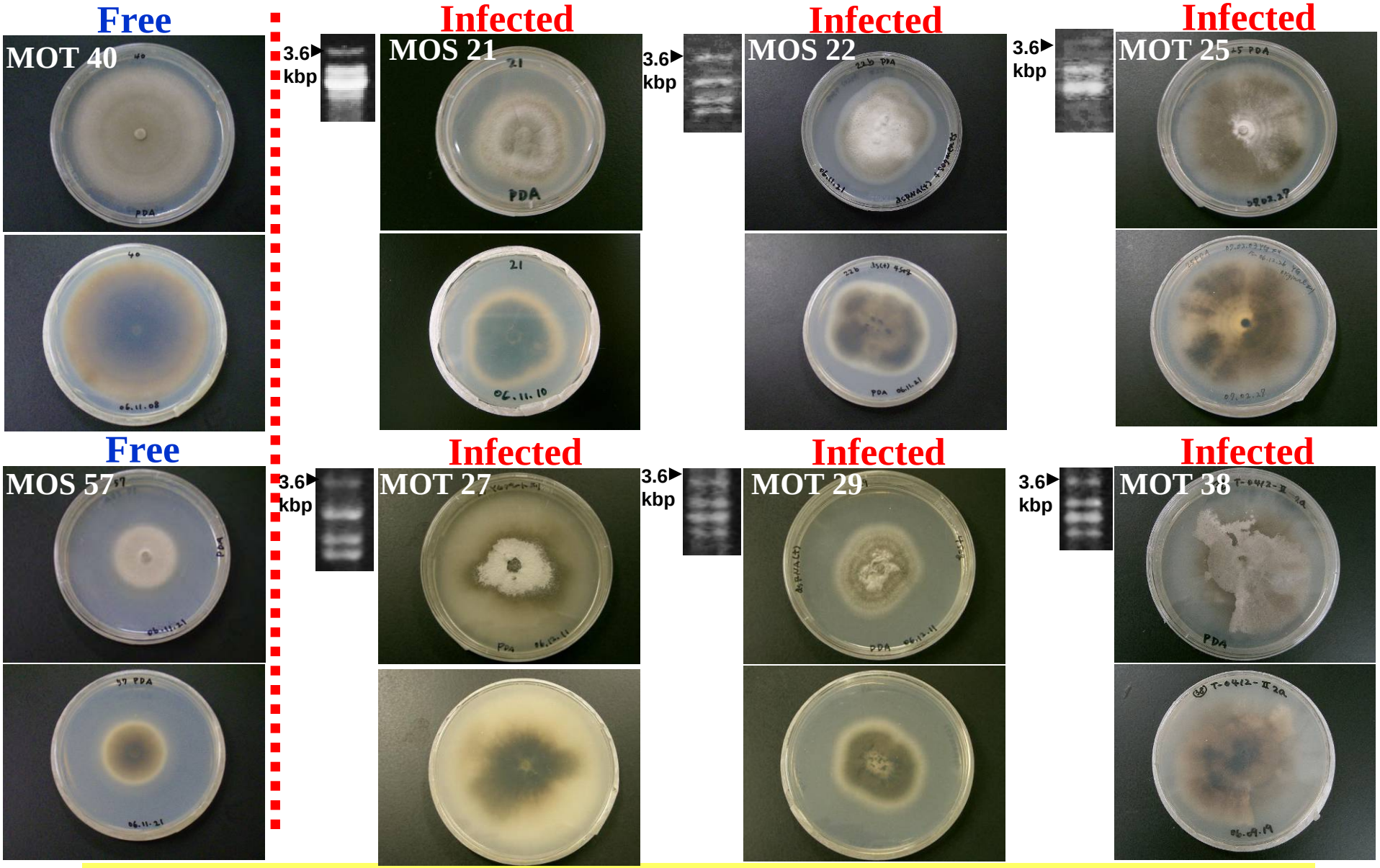
Virus family (virus name)	dsRNA genome		Virus particle	3' poly(A)
	Segment	Size(kbp)		
AaV	4	1.5-3.6	Isometric, 33nm	20-50 nt
Totiviridae	1	4.6-6.7	Isometric, 33-40nm	Not found
Partitiviridae	2	1.4-2.2	Isometric, 30-38nm	20-30 nt (Interrupted)
Crysoviridae	4	2.4-3.6	Isometric, 35-40nm	Not found

Conclusion

AaV seems to be related to the typical mycovirus families, *totiviridae, partitiviridae* and *crysoviridae*, but not classified into these families.

Mycoviruses Associated with Impaired Growth of the Rice Blast Fungus, *Magnaporthe oryzae*

Comparison of phenotypes between mycovirus -Free and -Infected strains, *Magnaporthe oryzae*



Mycovirus-infected strains showed abnormal growth phenotypes.

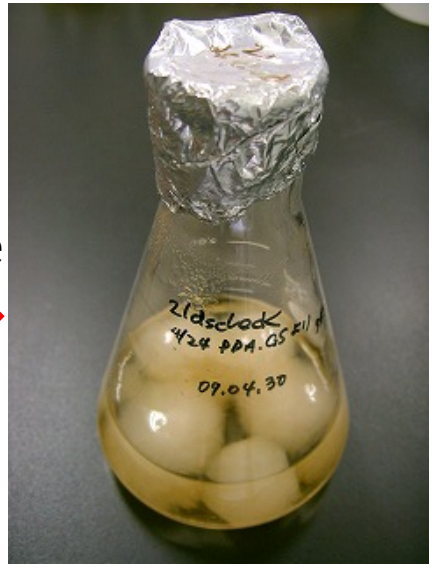
Isolation of viral dsRNAs from *M. oryzae*

Methods:

1. **Extraction of whole nucleic acids from fungal mats, then digestion with DNase I and S1 nuclease.**
2. **CF-11 column chromatography is sometimes performed.**

PDA medium
at 24 ℃ for 2weeks

Inoculate



YG liquid medium
at 24 ℃ for 2weeks

Harvest

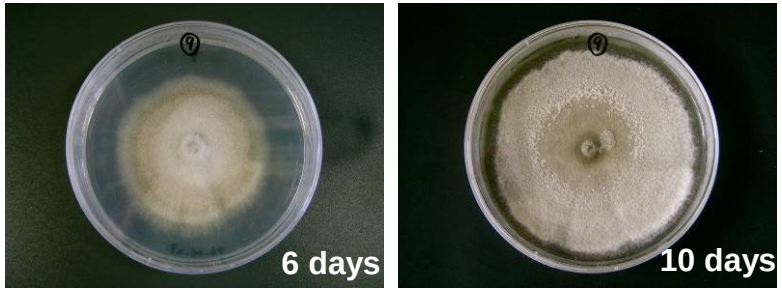
SDS-Phenol method
Enzyme digestion

Agarose gel electrophoresis

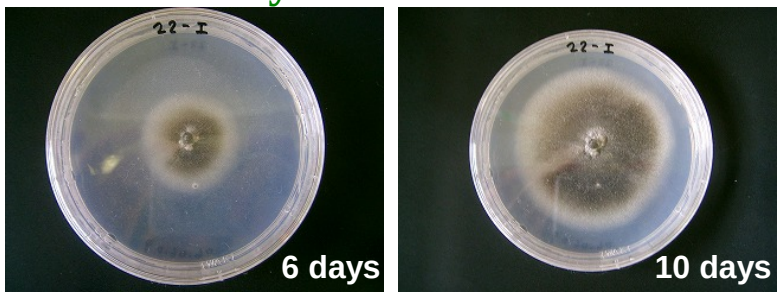
“dsRNA carring or not”

Comparison of growth rates between the mycovirus-infected strains and the free strains

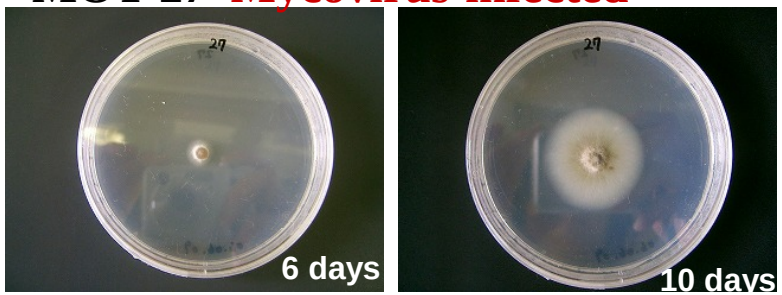
MOS 9 Mycovirus-free

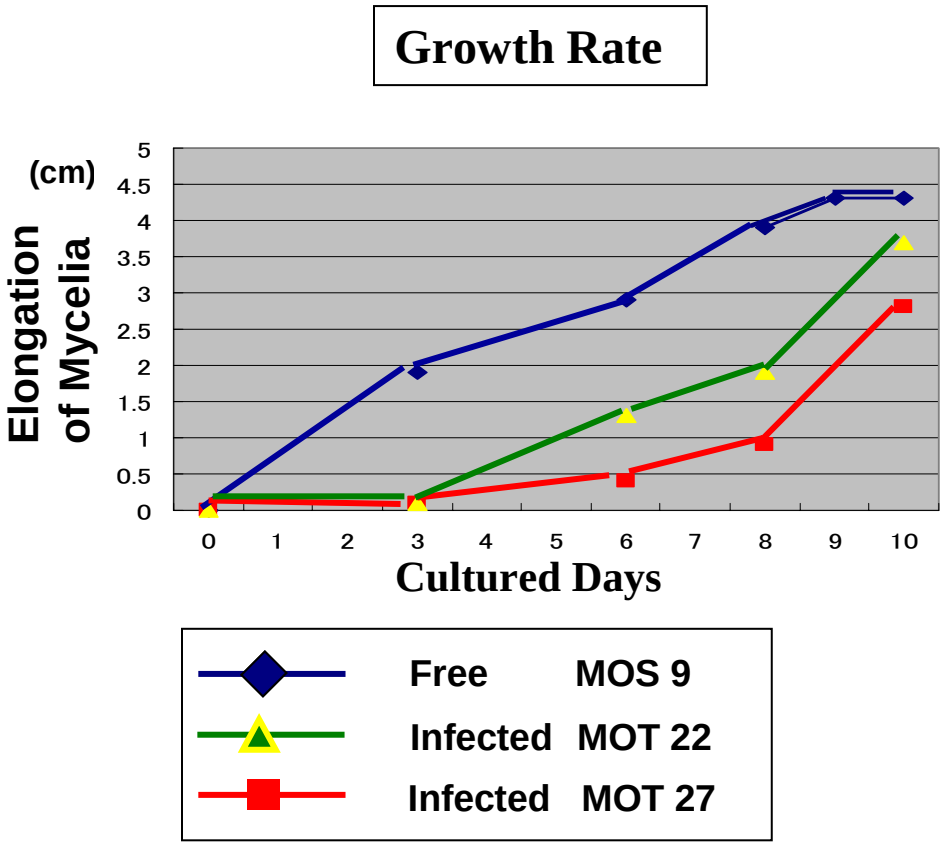


MOT 22 Mycovirus-infected



MOT 27 Mycovirus-infected



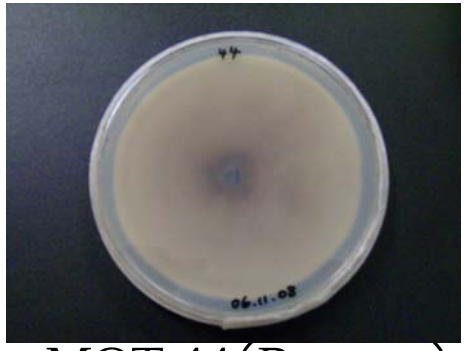


Mycovirus-free strains showed fast growth rates than the infected strains.

Phenotypes of mycovirus-Free and -Infected strains

Free strain

MOT 44（Surface）

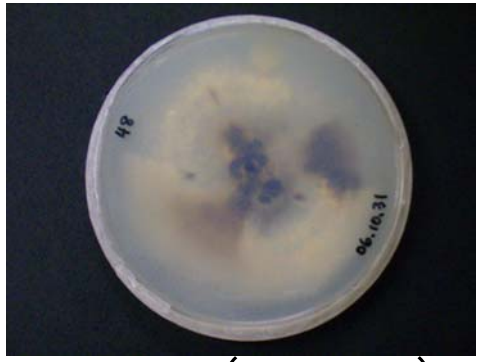


MOT 44（Reverse）

- Fast growth
- Regularly radial mycelia growth
- Normal pigmentation

Infected strain

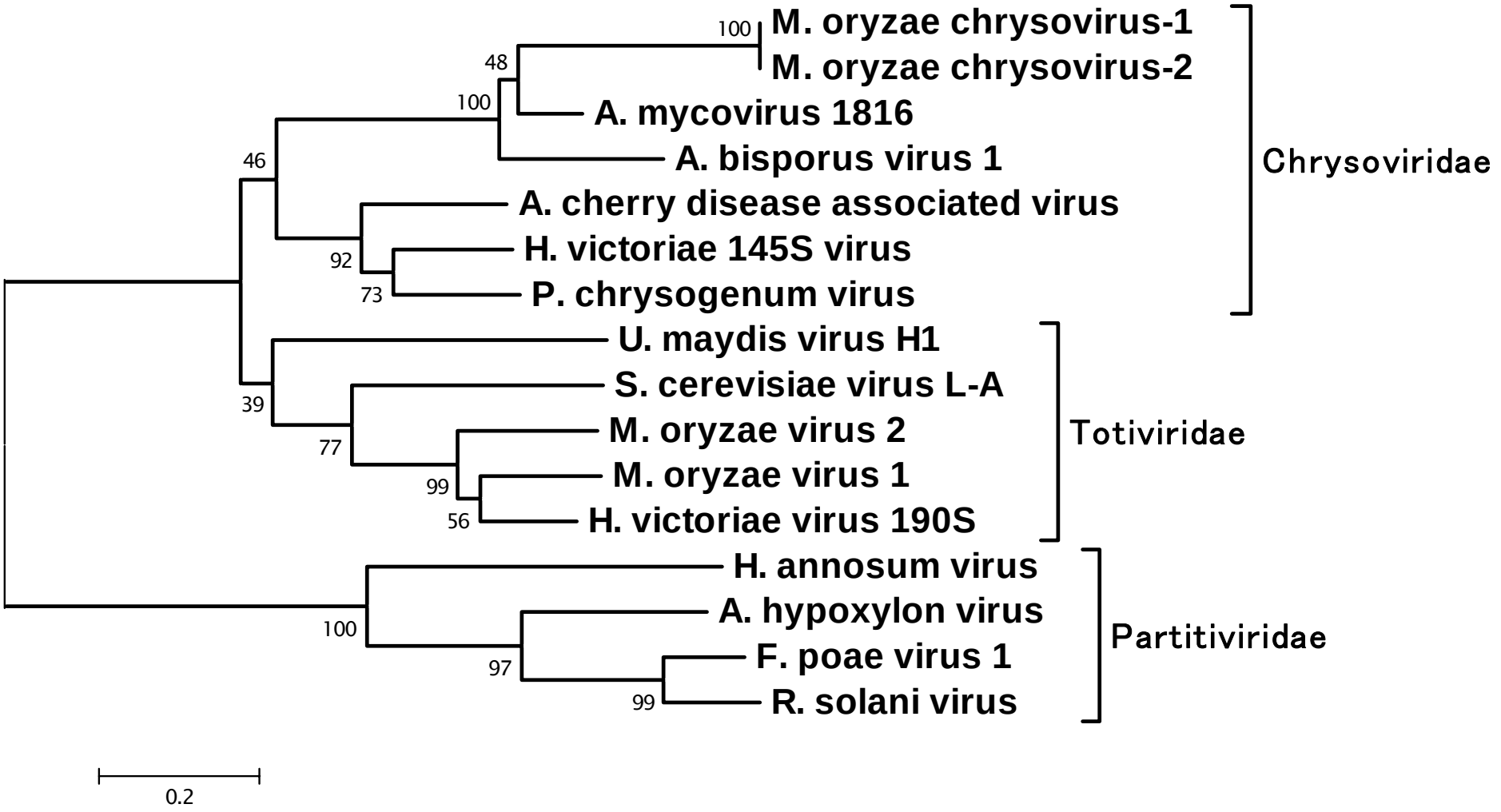
MOS 48（Surface）



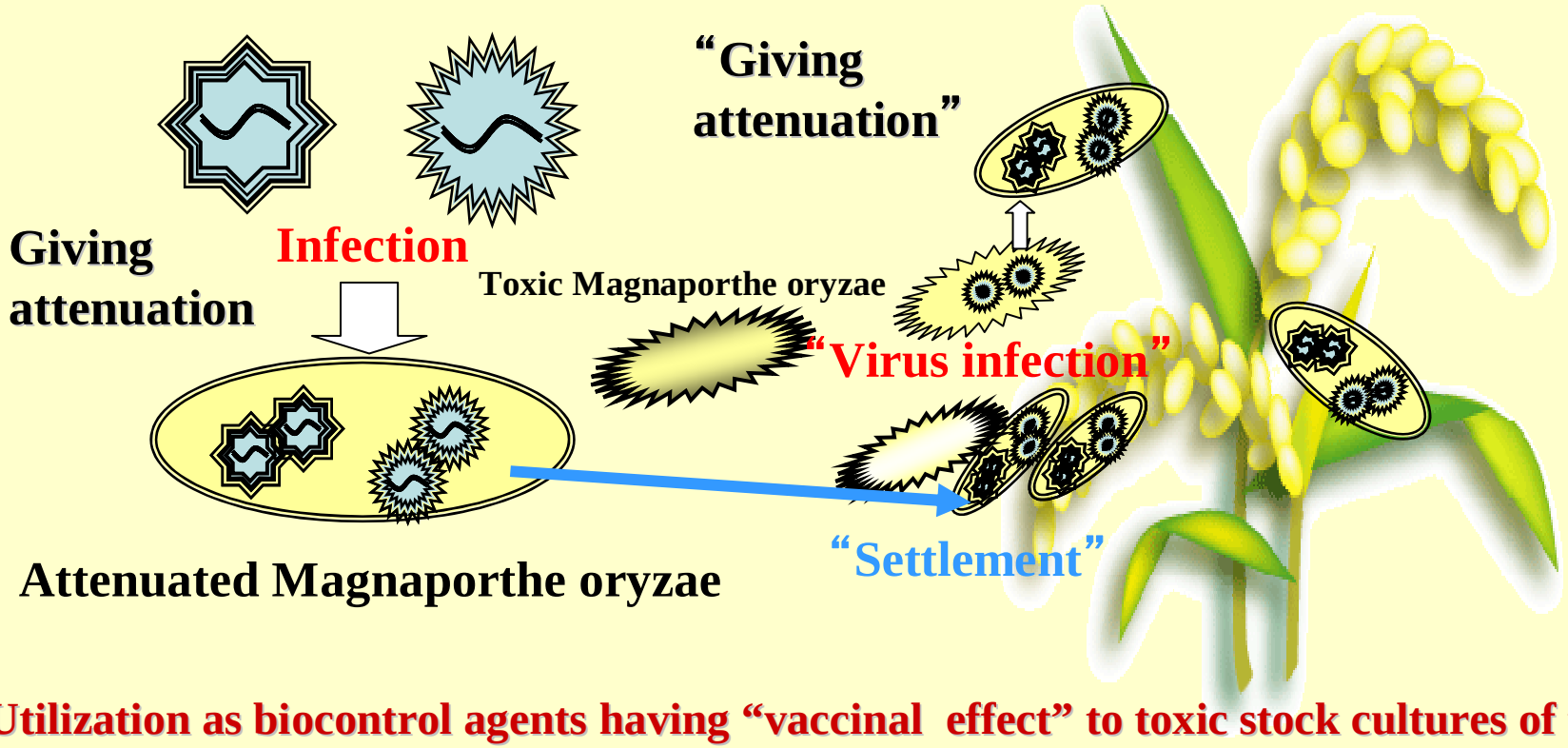
MOS 48（Reverse）

- Slow growth
- Irregular mycelia growth
- Irregular pigmentation
- Unusual aerial mycelium
- Autolysis

Phylogenetic analysis of the RDRP conserved motifs of MoCV1, 2 and selected totiviruses and partitiviruses



Final Target : Establishment of mycovirus infection method and preparation of stock cultures of fungi



Utilization as biocontrol agents having "vaccinal effect" to toxic stock cultures of fungi