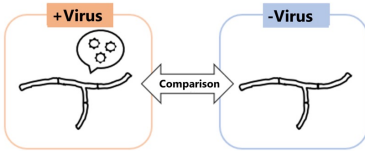


Background · Purpose

Mycoviruses have been reported to alter the phenotype of host fungi, including mycelial growth, sporulation, pigmentation, and virulence. However, in most cases, a virus is solely analyzed in each study, and comprehensive view by comparing multiple viruses was lacking. In addition, phenotypic analyses have not been systematically conducted, and most of the analyses have been conducted under limited test conditions, so the full picture of the effects of viruses on host fungi is unknown. To broaden our general understanding of mycovirus's nature, it is necessary to know the effects of multiple phylogenetically different viruses on host biology from multiple perspectives. Here, we conducted a systematic phenotyping of multiple viruses found in a single fungal species, *Aspergillus fumigatus*, to investigate the actual impact of mycoviruses on the host and to clarify the role of mycoviruses in the ecology of the host fungus.



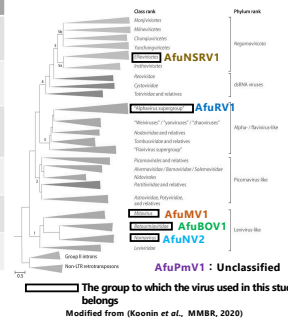
Material and Methods

► Mycovirus and the host strains

In our previous study, we identified 5 *A. fumigatus* strains that were infected with mycoviruses. The strains were preserved in Chiba university MMRC. (Chiba et al., *Virus Evol.*, 7, veaa101, 2020)

Strain	Virus
IFM63439	AfuRV1 (<i>Aspergillus fumigatus</i> ssRNA virus 1)
IFM62632	AfuPmV1 (<i>Aspergillus fumigatus</i> polyomyovirus 1)
	AfuNSRV1 (<i>Aspergillus fumigatus</i> negative-strand RNA virus 1)
IFM63431	AfuNV2 (<i>Aspergillus fumigatus</i> nanarnavirus 2)
IFM64916	AfuBOV1 (<i>Aspergillus fumigatus</i> botourmia virus)
IFM62355	AfuMV1 (<i>Aspergillus fumigatus</i> mitovirus 1)

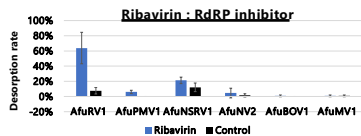
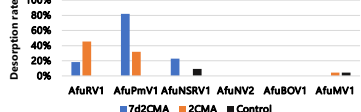
Phylogenetic tree of RNA viruses



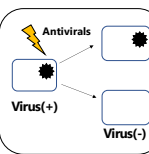
► Method to obtain virus-free strains

The strains were grown on agar media containing antiviral reagents (Ribavirin, 7d2CMA, or 2CMA). After one or several passages, the single-colony isolated strains were checked if the virus was eliminated by RT-PCR.

7d2CMA, 2CMA : Nucleoside analogues



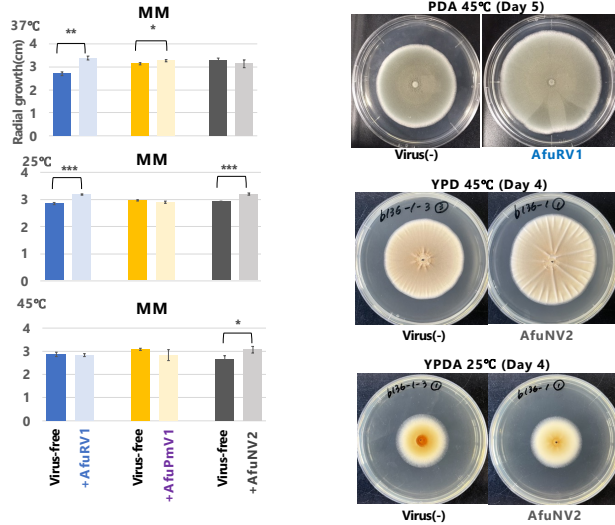
Virus elimination



Successful elimination of virus from the strains, which were used for the following phenotyping experiments.

Result : Effect of virus on colony growth and morphology

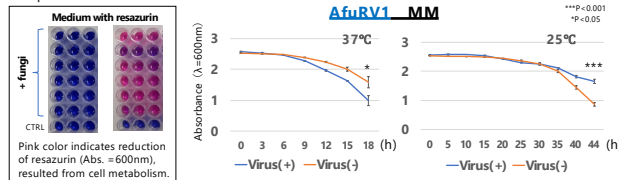
Conidia of the strains were inoculated on minimal media (MM), PDA, and YPD plates, and then incubated at 25, 37, and 45°C for up to 6 days.



Mycovirus affected the host's colonial growth and morphology. However, it depended on growth temperature, growth media, and virus species.

Result : Effect of virus on initial growth of host

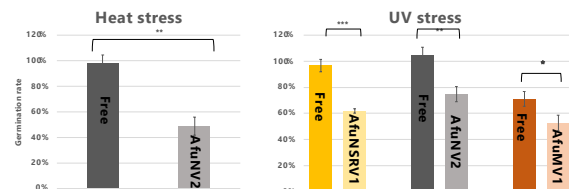
Fungal growth at germination stage was estimated by resazurin-based assay at different temperatures.



→ The virus affected host's germination in a manner dependent on culture temperature.

Result : Effect of virus on stress tolerance of the conidia

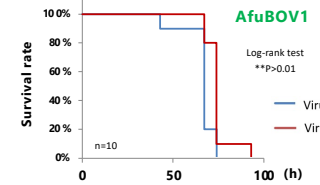
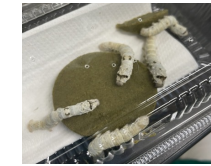
Germination rate was investigated upon heat treatment or UV irradiation. The conidia were subjected to the stresses, and then CFU was calculated.



The conidia of strains with virus infection showed higher sensitivity to heat or UV stresses.

Results: Effect of virus on host's virulence

Virulence over silkworm was compared between strains with and without virus infection.

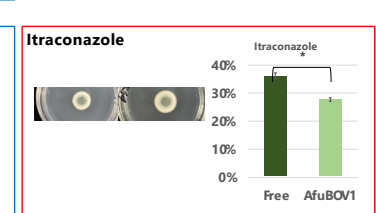
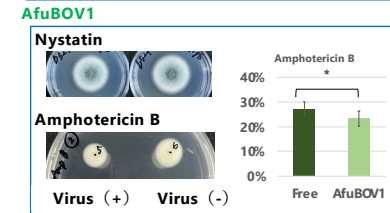
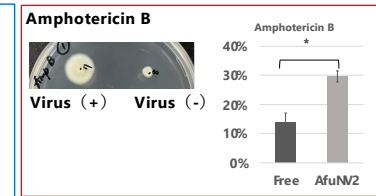
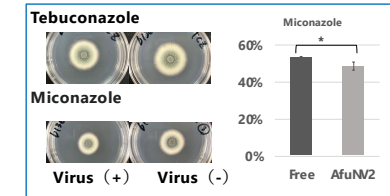


Fungal strain infected with AfuBOV1 showed stronger virulence compared with that without infection.

Results: Effect of virus on drug sensitivity

Colony diameters were compared on PDA plates with and without the drug indicated.

AfuNV2 Retention of the virus **reduces** resistance. **increases** resistance



	voriconazole	Itraconazole	Tebuconazole	Miconazole	Micafungin	Amphotericin B	Nystatin
AfuRV1		↑	↑↑	↑	↓↓		
AfuPmV1		↑↑					
AfuNSRV1							
AfuNV2	↓↓↓	↓	↓	↓	↓	↑	
AfuBOV1		↓	↓		↑	↓	↓↓
AfuMV1							

Drug susceptibility was altered by virus

Virus infection resulted in resistance ↑, susceptibility increased ↓
↑: P<0.05, ↑↑: P<0.01, ↑↑↑: P<0.001

Conclusion

- Viral function was examined in 5 strains of *A. fumigatus* infected by different mycoviruses comparing with the virus-free strains that were obtained by antiviral agents.
- The host's growth, conidia stress tolerance, virulence, and drug susceptibility were positively or negatively affected by mycovirus.
- Noteworthy, several mycoviruses are involved in multi drug susceptibility in *A. fumigatus*.

These results suggest that mycovirus can be regarded as a factor that shapes phenotypic diversity to the host fungi.