

A Review Paper on Mycoviruses

Aqleem Abbas*

College of Plant Science and Technology, HZAU Wuhan, China

Abstract

Mycoviruses are very significant viruses, which are found to be infecting fungi. These Mycoviruses require the living cells of their hosts to replicate like the plant and animal viruses. The genome of Mycoviruses mostly consist of double stranded RNA (dsRNA) and least of Mycoviruses genome consist of positive, single stranded RNA (+ssRNA). Moreover, DNA Mycoviruses have been reported recently. These viruses have been detected in almost all fungal phylum but still most of the Mycoviruses remain unknown. Mycoviruses are important in a sense that they mostly remain silent and rarely develop symptom in their hosts. Some Mycoviruses have been reported which are causing irregular growth, abnormal pigmentation and some are involved in changing their host sexual reproduction. For the management of Plant diseases, the importance of Mycoviruses arises because of their most significant effect that is they reduced virulence of their host. Technically the reduced virulence is called hypovirulence. This hypovirulence phenomena has increased importance of Mycoviruses because it has the potential to reduce the crop losses and forests caused by their hosts which are plant pathogenic fungi. In this review, I explore different aspects and importance of Mycoviruses.

Keywords: Mycoviruses; Genome; Hypovirulence; Evolution

Introduction

Mycoviruses are infecting fungal species and present in latent stage in them and rarely causing diseases [1]. There are few Mycoviruses which are involved in causing considerable changes in their fungal hosts but the most obvious changes in their fungal hosts are irregular growth, abnormal pigmentation and mutated sexual reproduction [1-4]. The most important quality of Mycoviruses, which can be utilized to manage fungal disease, is the hypo virulence, which means the reduction of virulence of plant pathogenic fungi. This quality has nowadays attracted much attention because several fungal diseases of forests as well as crops can be managed [4,5]. Research on these Mycoviruses, over the last fifty years has increased our know how regarding Mycoviruses.

Moreover, their interaction with their plant pathogenic fungal hosts has also been explored [2]. The first report of interaction was between Hypovirus CHV1 and the chestnut blight fungus *Cryphonectria parasitica*. The fungi *C. parasitica* produced abnormal pigmentation and reduced growth upon infection by CHV1 virus. We can say, the Mycovirus CHV1 induced Hypovirulence in chest nut blight [4,6]. Later several fungal species were studied which were infected with Mycoviruses. The other important plant pathogenic fungi was *F. graminearum* which has been widely studied [7]. The Mycovirus FgV1 induce hypovirulence to *F. graminearum* [8]. Upon infection with FgV1, the fungi *F. graminearum* exhibited reduced vegetative growth, abnormal pigmentation and mycotoxin [7]. More important aspect of mycovirus FgV1 is that, it can be transmitted to other fungal species such as *C. parasitica* where it induce more severe hypovirulence that its own Mycovirus CHV1 [9].

History of Mycoviruses

As previously mentioned, the Mycoviruses are the viruses, which are found to be in association with the fungal species. The first report on Mycoviruses appeared in 1962 from *Agaricus bisporus*. This mushrooms found to have misshaped fruiting bodies and consequently reduction in yield [2]. These Mycoviruses shared some of the characteristics with animal and plant virus but also have the distinct characteristics such as they lack an extracellular route for infection, transmitted intercellularly only through cell division, sporulation, and cell fusion and absence of a movement protein, which is essential for the life cycle of animal and

plant viruses. Taxonomists reported the genome of most Mycoviruses consists of double-stranded RNA (dsRNA), while the genome of about 30% of Mycoviruses is a positive, single-stranded RNA (+ssRNA) [8]. The DNA Mycoviruses related to group Gemini virus have been recently reported for the first time [3]. Mycoviruses have been detected in all the major fungal phyla including Zygomycota, Ascomycota, Deuteromycota, Basidiomycota and Chytridiomycota. Most of the Mycoviruses remain unknown. Currently transcriptomic approaches have been widely used for identification and detection of several Mycoviruses.

Evolution of Mycoviruses

The classification of Mycoviruses is same as the other viruses developed by ICTV. They have been grouped into following seven linear dsRNA families;

- Chrysoviridae
- Endornaviridae
- Megabirnaviridae
- Quadriviridae
- Partitiviridae
- Reoviridae
- Totiviridae

Moreover, they have been classified into five linear positive-sense ssRNA families

- Alphaflexiviridae

***Corresponding author:** Aqleem Abbas, College of Plant Science and Technology, HZAU Wuhan, China, Tel: 00862787281181; Fax: 00862787396057; E-mail: aqlpath@gmail.com

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- Barnaviridae
- Gammaflexiviridae
- Hypoviridae
- Narnaviridae

Moreover, there are some unclassified linear negative-sense ssRNA, and circular ssDNA virus [2].

These Mycoviruses have usually been detected upon purification of dsRNA molecules because many Mycoviruses produced dsRNA or dsRNA replicative intermediates in their fungal hosts [1]. The purified dsRNA when profiled exhibited high diversity of these Mycoviruses. Various dsRNA possessing fungal isolates exhibited multiple dsRNA patterns that might represent segmented viral genomes, also mixed infections of more than two viruses or defective RNAs have been explored [1,10]. The number of Mycoviruses whether dsRNA and/or ssRNA might be overestimated, however by studying phylogeny we have reached to conclusion that viruses in the same taxonomic families can infect diverse hosts such as fungi, plants, animals and protozoa [2,11]. Let us explain this by an example; the current taxonomic review suggested that the family Partitiviridae contained dsRNA viruses that infect plants, fungi, or protozoa [12]. Mycoviruses such as *Cryphonectria parasitica* Hypovirus 1-4 (CHV1-4), *Fusarium graminearum* virus 1 (FgV1), and Botrytis virus X, are phylogenetically related to other plant viruses. When investigated their genome, it was found that their genomic organization and expression strategy is similar to those of plant poty viruses or potex like viruses [2,13]. The other virus such as RNA virus L of *Sclerotinia sclerotiorum* is closely resemble to the human pathogen hepatitis E virus and Rubi like [3]. For the origin of Mycoviruses two major hypothesis have been proposed [1]. One of the hypothesis is called the “ancient coevolution hypothesis” which states that the origin of Mycoviruses is still remain a mystery, and reflects a long term coevolution. The other hypothesis is known as plant virus hypothesis, in contrast, which states that Mycoviruses originated relatively recently from the other plant viruses i.e. the original mycovirus was a plant virus that drifted from the plant to fungus within the same host plant [12]. Still both the hypothesis are not convincing, so the origin of Mycovirus remains a mystery. There are other hypovirulence associated Mycoviruses such as a) *Sclerotinia sclerotiorum* hypoviruses b) *Helminthosporium victoriae* viruses c) *Rosellinia necatrix* viruses. These Mycoviruses have been detected and studied using reverse genetic approaches [1,5]. These Mycoviruses have deficiency of extracellular phase, so researchers have investigated various transfection methods using the purified virus particles, full-length viral cDNA clones and *in vitro* RNA transcripts [14-16]. These infection assays were helpful in identification of the viral and/or host factor(s), which are involved in symptom induction or virus replication of several Mycovirus host interactions. These techniques can also be utilized to explore the host ranges of several Mycoviruses. As previously mentioned, these Mycoviruses are transmitted intercellularly via hyphal anastomosis or spores. The transmission of viruses between different strains is inhibited by fungal vegetative incompatibility (vic). Vegetative incompatibility is one of key obstacle in the use of hypovirulent Mycoviruses used as biocontrol agents. The current research has opened a gate by demonstrating that seven vic genes which are present in five or six loci in fungal species *C. parasitica* played a role to incompatibility and also affect virus transmission [17]. Moreover, much of the research concerning Mycoviruses has dealt with hypovirulence of plant pathogenic fungi but several dsRNA and ssRNA viruses have also been reported from the yeast *Saccharomyces cerevisiae*

[18]. The most important viruses among them is *Saccharomyces cerevisiae* virus L-A (ScV-L-A) which has been well characterized.

Interaction of Mycoviruses with their Fungal Hosts

RNA Genome expression analysis revealed that there are several expression patterns of *F. graminearum* transcriptomes infected by four phylogenetically different Mycovirus [19]. Among these different Mycoviruses, the virus FgV3 and FgV4 did not cause any obvious changes in the phenotypes of their host. While the other viruses such as FgV1 and FgV2 which caused some obvious changes in the host phenotypes [19]. Moreover, the detailed study will finally enhance our knowledge of the interactions among these Mycoviruses and their fungal hosts. Mycoviruses are obligate intracellular parasites; they are involved in reprogramming of their host metabolism. They also involved in antiviral responses. There we can only know their role in the life cycle of fungal host if we would be able to identify the important determinants which play their role in their interaction [20]. To identify these determinants currently scientist, use wide genome approaches particularly in studies of Mycoviruses of *Cryphonectria*, *Fusarium graminearum* and *Sclerotinia sclerotiorum* [19-22]. The results showed the expression level of fungal genes differed between virus free and virus infected fungal isolates. Moreover, they are also found to be different among the viruses belong to different groups. Further, the results indicate that these Mycoviruses not only depend on various host factors but also on cellular processes and pathways such as related to cellular transport, metabolism, RNA processing and other RNA signaling.

In viruses of Yeast such as ScV-L-A, the host protein Mak3p which is an N-acetyl transferase is essential for the acetylation of major coat protein of that viruses and such acetylation is also very important for the virus assembly [18]. Quelling in fungi also termed as RNA silencing [23,24], has also been investigate with the fungi *Neurospora crassa*, and other reports are also found for *C. parasitica* and *Aspergillus nidulans* [24]. There is a large potential in the genome of Mycovirus because they consist of RNA, and thus quelling can protect their host from other Mycoviruses. The genes dc12 and ag12 are very important for RNA-silencing for CHV1 infection in *C. parasitica* [24]. Some silencing-related genes (rdr1, dcl1, dcl2, and agl2) upon virus infection were also observed in *Fusarium graminearum* viruses-infected *F. graminearum* [19]. Many viruses have then devised a different strategy to suppress RNA silencing for example the p29 protein of Mycovirus CHV1 and the S10 gene product of *Rosellinia necatrix* Mycoreovirus function as silencing suppressors [6,25]. The host genes that have been analyzed for biological function in mycovirus-host interactions are shown in Table 1.

Future Challenges in Studying Mycoviruses

Much of the early research on Mycoviruses has put them undesirable because they were attacking commercial mushrooms. Later they have been found to be considered beneficial when they were found to be act as biocontrol agents of fungal pathogens in the most economically important plants [3-5]. The first approach to manage diseases was carried out in the 1980s, the spores of fungi *C. parasitica* containing Hypovirus were artificially introduced into fungal populations to control chestnut blight. This approach completely became unsuccessful in orchards in eastern North America and in Europe [4]. The variation in efficacy resulted from the vegetative compatibility among fungal isolates or from the features of the hypoviruses [17]. These results suggests that the use of Mycoviruses as effective biological control agents may further require consideration of several factors including

Fungus	Fungal host gene	Mycovirus	Function involved in	Ref.
<i>C. parasitica</i>	Pro1	CHV1	Stable inheritance of CHV1	[26]
<i>H. victoriae</i>	Bir	CHVs	Transmission of the hypoviruses	[27]
<i>S. sclerotiorum</i>	MK1, PK1	CHV1	CHV1 symptom development	[28]
<i>F. graminearum</i>	NAM-1	CHV1	CHV1-induced symptom expression	[29]
	Hv-p68	HV145S	Accumulation of Hv145S	[30]
<i>S. cerevisiae</i>	SsITL	SsDRV	Suppression of host resistance for SsDRV	[31]
	FgHex1	FgV1	Accumulation of FgV1 viral RNA	[23]
	FgHal2	FgV1	Accumulation of FgV1 viral RNA, transmission of FgV1	[32]
	Mak3	ScV-L-A	ScV-L-A assembly	[18]
	Ski1	ScV-L-A	Degradation of ScV-L-A viral RNA	[18]

Table 1: The host genes that have been analyzed for biological function in mycovirus-host interactions.

both the host and virus properties. Recently the *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 was used to manage the rapeseed stem rot disease and this hypovirulence reduced the disease. The virus was applied as a suspension of virus-infected hyphal fragments or virus particles [3,5]. Several challenges are ahead in the use of hypovirulent strains to control plant pathogenic fungi. The challenges are as follows

- Vegetative incompatibility, which inhibit the transmission of Mycovirus from a hypovirulent strain to a target strain
- The potential lack of fitness of the hypovirulent strain
- Most of the Mycoviruses do not significantly affect their fungal hosts. These suggests that these viruses may be adapted to living with their host for the long period of time. This kind of association might be helpful for both Mycovirus and its host
- The “killer phenomenon or killer hypothesis” is also a new phenomenon in the yeast. This is caused by combined presence of cytoplasmically inherited dsRNA virus and satellites or DNA virus-like elements (VLEs) [18]. Although these viruses do not induce symptoms in their hosts, they do substantially affect host biology

Conclusion

- The scientists are struggling to understand the Mycoviruses but still don’t know how to initiate infection so as to determine cause and effect of the Mycoviruses associated with their fungal hosts.
- The trans-infection methods and reverse genetic systems have been developed for several Mycoviruses. The future reverse genetic systems might help us to understand the molecular biology of Mycoviruses and used them as stable biological control agents or as virus based expression vectors.
- Moreover, the future studies are probably revealing the important clues of Mycoviruses which will be helpful in understanding their role in fungal hosts. Further the research on Mycoviruses and their fungal hosts will provide new insights into the largely dark matter of Mycoviruses

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