

Fungal Viruses: Promising Fundamental Research and Biological Control Agents of Fungi

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Abstract

Fungal viruses (mycoviruses) exist in all major groups of fungi. The primary focus of this review is viruses of filamentous fungi, especially plant pathogens, with emphasis on the molecular characterization of fungus-virus interactions. There are two main hypotheses about the origin of mycoviruses isolated from plant pathogenic fungi. The origin of these mycoviruses may be ancient but they may also have evolved from plant viruses. Many characterized mycoviruses are in unencapsidated dsRNA forms without any coat protein in fungi. Mycoviruses are efficiently spread in two ways, vertically by spore formation and horizontally via hyphal fusion. Replication cycle of RNA mycoviruses doesn't have the extracellular route of infection under natural conditions. Typically, fungal infections cause no obvious phenotypic alterations. Although the interaction of mycoviruses and their host is largely limited, those aspects including the transcriptional profiling and RNA silencing are of help to understand the co-existence mechanism of virus and fungi. Mycoviruses are potential agents of biological control of important plant pathogenic fungi.

Introduction

Viruses that selectively infect fungi are named mycoviruses. Mycoviruses, which were discovered late, haven't got the same attention as plant and animal viruses probably because most mycoviruses cause symptomless infections. The first report on mycovirus was published in 1962 [1] but to date, they have been isolated from all major taxonomic groups of fungi. Mycoviruses are mainly discovered by nucleic acids isolation and sequencing and characterized by comparisons between mycovirus-infected and free fungal strains. The origins of mycoviruses remain uncertain and some hypotheses are proposed. Our knowledge of mycoviruses is slowly but steadily accumulating.

Mycoviruses have various genome types, including double-strand RNA (dsRNA), single strand RNA (ssRNA), single strand DNA (ssDNA). In the 7th International Committee for Taxonomy of Viruses (ICTV) report, only two ssRNA mycoviruses were reported. By far over a third of identified mycoviruses are characterized by ssRNA genome type. Many previously characterized dsRNA mycoviruses are supposed to be ssRNA mycoviruses at present. Generally, it's accepted that dsRNA extracted from hypha is the replicative intermediate or replicative form of an ssRNA virus. According to the 9th report of the ICTV on virus taxonomy lists, mycoviruses are currently classified into seven dsRNA families, six ssRNA families and circular ssDNA (unclassified). Mycoviruses are being discovered at an increasing rate but many of them still remain unclassified.

Isometric particles are observed for most mycoviruses that encode coat proteins. However, many mycoviruses are naked viruses with an absence of particle morphologies. Since RNA mycoviruses don't have an extracellular route of infection, the efficiency of their transmission was limited by vegetative incompatibility. Although many efforts are put on the screening and sequencing of novel mycoviruses, increasing researchers have begun to attempt to illuminate the interactions between mycoviruses and fungi in recent years. In this paper, we emphatically review the interactions between mycoviruses and host fungi. Some potential competing mechanism in the co-existence of fungi and viruses has been illustrated. Currently, most mycovirus research papers are concerned with cultivated mushrooms, yeasts and plant pathogenic fungi that are economically important. To our knowledge, some hypo virulent mycoviruses have been used as a tool to combat plant pathogenic fungal diseases. Even there are still some limitations; mycovirus therapy definitely, represents a promising direction for biological control of fungal diseases.

Mycoviruses Evolution

The origins of mycovirus have always been different, since there is no single argument. Virologists have advanced two hypotheses to deduce origins of mycoviruses. The first hypothesis

is that mycoviruses are of ancient origin and coevolved together with their fungal hosts. This hypothesis is based on the proposal that RNA mycoviruses don't have any extracellular transmission routes. Individual mycoviruses are generally limited to a single host species and it's difficult to transmit mycoviruses among different fungal species. Mycoviruses and their hosts have developed a co-evolutionary relationship over a long period. Another evidence is that some mitochondrial mycoviruses have been proved to use host mitochondrial genetic translation codes in *Chalara elegans* [2]. In these cases, such as the killer systems in yeast, mycoviruses are beneficial to the host, acting as extra-chromosomal [3]. As we know that most characterized mycoviruses cause symptomless phenotype, which may be the results of a long period of natural selection. Consequently, the vast majority of mycoviruses are at least not harmful and possibly beneficial for their fungal hosts.

The second hypothesis is that mycoviruses originate from plant viruses. This hypothesis is based on the sequence comparisons between mycoviruses and plant viruses. Several recently described mycoviruses are phylogenetically related to plant viruses [4-6]. Based on the sequence alignment and genetic analysis, these mycoviruses are clustered into the families that predominantly contain plant viruses. Some identified hypoviruses are phylogenetically related to potyviruses. These close phylogenetic relationships raise the possibility that at least some mycoviruses may have originated from plant viruses. It's possible that the viruses move into fungi in the process that the fungus infects the virus-carrying plant. Although the incidence of internalization may be rare, the possibility could not be overlooked. However, it's also possible that part of plant viruses derived from fungal viruses. By using the new sequencing technology, an increasing number of mycovirus genome sequences are being published, which will help our understanding of mycovirus evolution based on phylogenetic relationships.

Transmission of Mycoviruses

Normally, the incidence of mycoviruses in filamentous fungi is determined by the presence of dsRNAs. It's reported that the incidence of mycovirus in different fungi species varies from a few percent to 100% [7-9]. Usually, a specific mycovirus is found for a particular fungal species, which is different from plant or animal viruses. However, there are exceptions like *Cryphonectria hypovirus 1* (CHV1) which has been identified in several *Cryphonectria* species. In different species of ascomycetes and basidiomycetes, similar viruses are detected [10,11].

Mainly, Mycoviruses spread by hyphal fusion or sporulation. And, extracellular routes were not observed for RNA mycoviruses in their natural life cycle. There are two major intracellular transmission ways: vertical and horizontal transmission. Vertical transmissions through asexual and sexual spore formation are primary means of mycovirus spread. Normally, most mycoviruses are highly efficiently transmitted to asexual sporulation. The transmission rate nearly reaches 100% via asexual spores in some cases [12]. Transmission rate through sexual spore types varies greatly and is usually lower than that of asexual transmission. In many cases, mycovirus particles or naked genomes are found in the cytoplasm. In the process of cytoplasmic exchange of fungal cells, mycoviruses spread into uninfected mycelial cells. According to this theory, mycoviruses are transmitted during cell fusion, division and mating with vegetative compatible strains.

The vegetative incompatibility is the main barrier to mycovirus horizontal transmission. When hyphae of two incompatible fungal strains fuse, they recognize each other as non self causing the fusion cells death, a type of Programmed Cell Death (PCD). The cytoplasmic exchange fails, resulting in impossible mycovirus horizontal transmission. By using molecular techniques such as transformation with cDNA infectious clone or RNA transcripts and protoplast fusion in special experimental settings, researchers have transfected various mycoviruses into different incompatible fungal strains. However, in some cases, mycoviruses can transmit to genetically incompatible fungal species under natural conditions. The rejection of heterokaryon is formed mildly with the presence of mycoviruses. It's was believed that some mycoviruses may counteract PCD via suppression of genes involved in PCD activation.

Symptoms of Mycoviruses

Typically, mycoviruses cause cryptic or latent (symptomless) infections. Some mycoviruses can cause negative effects, including altered colony morphologies, reduced sporulation and growth rate and attenuation of virulence in their host. Mycoviruses that can attenuate the pathogenicity of fungi cause a great deal of interest for their potentiality of biological control. In particular, some kinds of yeasts, including *Saccharomyces*, *Hanseniaspora* and *Zygosaccharomyces*, and *Ustilago maydis*, can encode lethal toxin [13]. These toxin-secreting "killer yeasts" can kill the sensitive yeasts strains around, and thus get more nourishment. Killer toxin-encoding mycovirus have also been isolated from a filamentous fungus that exhibits cytotoxic to mycovirus-free strains [14]. Perhaps more interesting, some special mycoviruses are beneficial to their hosts [15]. It has been reported that a mycovirus in the endophytic fungus *Curvularia protuberata* has beneficial effects on the host plant, panic grass *Dichanthelium lanuginosum* by improving the plant's ability to withstand high temperature [16]. It's also worth to note that two CHV1 hypovirus isolates, CHV1-EP713 and CHV1-Euro7, which share high sequence identities, have distinct symptom profiles.

Mycoviruses Fungi Interactions

The study of interactions between mycoviruses and fungal host started late relative to plant or animal virus-host system. However, there are some advantages using the virus-fungi model to explore virus-host interactions more broadly. The fungal genetics are much simpler, especially when compared with those of *Arabidopsis thaliana* or *Nicotiana benthamiana*. Although the genetic manipulation is not well developed in fungi, advances in the technology come quickly. Another advantage is, most obviously, that fungi have much shorter culture cycle than plants or animals.

Transcriptome profiling is an alternative method to reveal the potential genes or pathways that are responsible for symptoms induction and gene alterations under virus infection. There is a fair amount of research on *C. parasitica*-mycovirus model about fungi-virus interactions. With the mRNA differential display technology, transcriptome changes between CHV1 infected and virus-free *C. parasitica* strains were compared [17]. A cDNA microarray of *C. parasitica* has been used to explore the changes induced by hypovirus infection at the transcriptional level [18]. A series of *C. parasitica* genes were regulated, including carbon metabolism, transcriptional regulation and stress responses. Gene expression comparisons between *C. parasitica* strains infected with CHV1-EP713 and CHV1-

Euro7 were also performed. The expression differences were especially striking though CHV1-Euro7 shares a high level of nucleotide and amino acid identities with CHV1-EP713.

Beyond the *C. parasitica* mycovirus model, a number of transcriptome profiling has been characterized in other fungi-mycoviruses systems. In *Sclerotinia sclerotiorum* debilitation-associated RNA virus (SsDRV)-infected *S. sclerotiorum*, 150 genes was expressed differentially compared to the SsDRV-free strain. These genes were related to protein synthesis and transport, stress response, and so on [19]. Protein and transcription expression levels were compared between FgV1-infected *Fusarium graminearum* and virus-free strain. Genes involved in various pathways including protein synthesis and cAMP signaling were enriched [20,21]. By using the NGS technology, 12 differentially expressed genes were commonly identified in response to all four mycovirus infected *F. graminearum* [22]. Wang *et al.* found that cellular redox regulation was one of the main stress response in FgHV1 infected *F. graminearum* by transcriptome-based analysis. In addition, FgHV1 encoded p20 could induce hypersensitive responses in vitro [12]. Besides, the transcriptome levels of G-protein and mitochondrial function disruption related genes were also changed in mycovirus infected fungi [23,24].

RNA silencing is an important pathway of the regulation system in eukaryotic cells. It is the main antiviral defense response in viruses infected organisms. However, the deeply-research fungal species is *Neurospora crassa* in the aspect of RNA silencing system. Unfortunately, no viral system is established in *N. crassa* for lack of available mycoviruses. It has been demonstrated that RNA silencing acts as an antiviral defense mechanism in fungi [25]. In *C. parasitica*-CHV1 model, 171 CHV1-derived siRNAs were detected and sequenced [26]. Two Dicer proteins, four argonaute proteins and four RdRp proteins have been confirmed in *C. parasitica*. It had been demonstrated that *dcl-2* and *agl-2* genes played critical roles in antiviral RNA silencing process. In some cases, RNA silencing could have a positive or negative effect on the frequency of viral genome rearrangements [27,28]. By using NGS, 1,831,081 and 3,254,758 FgHV1 and FgHV2-derived small RNAs were identified in *F. graminearum*, respectively indicating that viral RNA trigger RNA silencing in fungal host. Host miRNA expression profiles were also changed by FgHV1 infection in *F. graminearum* [29].

Viruses use different strategies to escape host RNA silencing in plant and animal viruses [30]. One of interest is coding RNA silencing suppressors (RSSs). In plant viruses, many RSSs have been identified. These RSSs are diverse in sequences and structures and they display different host RNA silencing suppression mechanisms [31]. So far only two RSSs were identified in mycoviruses group. CHV1 encode continuous two open reading frames, ORF A and ORF B. Poly peptide, p29 released from ORF A, was responsible for host RNA silencing suppression [32]. The second mycovirus suppressor was encoded by *s10* gene of *Rosellinia necatrix* mycoreovirus 3 which was classified into *Reoviridae* family [33]. Our work on *Agrobacterium* transient expression assays in *N. benthamiana* suggests that p20, encoded by FgHV1, is also an RSS (S.C.W, L.H.G unpublished results). More interesting still, host core RNA silencing genes, *FgDicer1* and *FgRdRp5*, were predicted to be targets of virus-derived sRNAs, which may be a novel anti-RNA silencing strategy employed by mycoviruses [29].

In recent years, *F. graminearum*-mycovirus has also been developed as a good model to explore fungi-virus interaction. The genome of the *F. graminearum* strain PH-1 was sequenced and published, which will provide the necessary genetic information for further study [34]. *F. graminearum* RNA silencing components, two dicer proteins, two argonaute proteins and five RNA-dependent RNA polymerases were characterized, among which FgDicer2 and FgAgo2 played critical roles in gene silencing process [35]. Till now, more than 10 mycoviruses has been discovered in *Fusarium* species (Table 1), providing sufficient resources for fungi-virus interaction study. Moreover, Lee *et al.* transfected four different mycoviruses into *F. graminearum* PH-1 strain making comparisons of these mycoviruses possible [22].

Promising Agents of Gene Vectors and Biological Control of Pathogenic Fungi

Nowadays, as many mycoviruses have little effect on their host fungi, they can be used as gene insertion vectors. Coat proteins of several plant viruses have been genetically modified to express human or animal's antigenic epitopes for vaccine development. For example, flexivirus *Potato virus X* has been used as vectors to express different kinds of genes in plants [45]. It's possible that the ssRNA mycoviruses belonging to the *Flexiviridae* family such as BVX, BCVF and SsDRV,

Table 1: List of mycoviruses identified from *Fusarium* species.

Name	Genome	Taxonomy	Symptoms in Fungal Host	Ref
FgV1	dsRNA	Suggested <i>Fusariviridae</i>	Hypovirulence, colony morphology alterations, mycotoxins reduction and sexual and asexual development disorders	[36]
FgV2	dsRNA	<i>Chrysoviridae</i>	Hypovirulence, colony morphology alterations, and sexual and asexual development disorders	[37]
FgV3	dsRNA	<i>Totiviridae</i> or <i>Chrysoviridae</i>	No effect	[38]
FgV4	dsRNA	<i>Partitiviridae</i>	No effect	[38]
FgV-ch9	dsRNA	<i>Chrysoviridae</i>	Hypovirulence, colony morphology alterations and asexual development disorders	[39]
FgHV1	ssRNA	<i>Hypoviridae</i>	Growth rate and asexual spore production reduction	[40]
FgHV2	ssRNA	<i>Hypoviridae</i>	Hypovirulence, colony morphology alterations, mycotoxins reduction and asexual development disorders	[41]
FgMTV1	dsRNA	<i>Tymoviridae</i>	Colony morphology alterations, mycotoxins reduction	[42]
FpV2	dsRNA	Unassigned	No characterization	[43]
FpV3	dsRNA	Unassigned	No characterization	[43]
FgDFV1	ssRNA	Suggested <i>Deltaflexiviridae</i>	No characterization	[44]

may be the candidates for gene insertion vectors. Likewise, one possible scenario is that engineering of genes involved in mycovirus can also lead to vaccine development.

Fungicides are relatively cheap, quick and effective at controlling some plant diseases. However, their applications are limited by many concerns including environmental pollution and resistance. Biological approaches are thus needed to assist in the control plant pathogens. It's intellectual and ecological to use mycoviruses to control fungal diseases. Unfortunately, most mycoviruses cannot be directly used in the field. However, the mycovirus CHV1 has been successfully used to control chestnut blight pathogen *C. parasitica* in Europe [46]. And this successful biological control of *C. parasitica* has inspired more mycoviologists to find novel mycoviruses with huge application potentials.

Fungal vegetative incompatibility shown by many species is likely to be the major barrier. Normally, hyphal fusion is the main way for mycoviruses transmission among different host species. It's hard for mycoviruses spread among natural host populations whose species are quite complicated. But the surprising and encouraging things are that purified *S. sclerotiorum* hypo-virulence-associated virus-1 (SsHADV1) particles can be directly used to infect *S. sclerotiorum*, thus making application of SsHADV1 as a spray available [47]. Another example is that *S. sclerotiorum* partitivirus 1 (SsPV1) can overcome vegetative incompatibility and transmit between mycelial incompatible strains [48].

It has been demonstrated that sprayed long dsRNA can be absorbed into fungi cells and used as biological control agents [49,50]. As we know, many mycoviruses have dsRNA or dsRNA replicative intermediate. So these mycoviruses that reduce the pathogenicity of fungi may be used as agents for dsRNA application in the field. Moreover, nanoparticles have been developed as dsRNA carrier for protection against plant viruses [51]. Nanotechnology has considerable promise for mycoviruses application as biological control agents in the near future.

Conclusions

Mycoviruses exist in all major groups of fungi. However, they have not caused enough attention. This review article summarized the development of mycoviruses from five aspects as discussed above, containing main contents for mycoviruses. Molecular characterization of fungus-virus interactions, which were discussed in detail, indicating mycoviruses are promising agents of fundamental research. We also put forward a new viewpoint on the application of mycoviruses: nanotechnology may be a revolutionary new tool for mycoviruses application in the field.

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