Brief Report

Mycoviruses are common among different species of endophytic fungi of grasses

Noemí Herrero, Salud Sánchez Márquez, Iñigo Zabalgogeazcoa

Instituto de Recursos Naturales y Agrobiología, CSIC. Apartado 257, 37071 Salamanca, Spain

Corresponding author: Iñigo Zabalgogeazcoa.
Email: i.zabalgo@irnasa.csic.es
Phone: +34 923219606
Fax: +34 923219609

Abstract

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Mycoviruses are common among different species of fungal endophytes of grasses.
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A survey of mycoviruses was made in a collection of 103 isolates belonging to 53 different species of endophytic fungi of grasses. DsRNA elements were detected in isolates of 12 of the species analyzed. The banding characteristics and sizes of some of the dsRNA elements suggest that they might belong to previously described mycovirus families. The observed incidence (22.6%) indicates that the presence of mycoviruses could be common among species of this group of ubiquitous fungi.

Endophytes are a group of fungi which infect aerial or underground plant parts without causing any apparent symptoms in their hosts. Endophyte surveys carried out in the last twenty years indicate that most, if not all plant species, are hosts of these fungi. Furthermore, numerous endophytic species, sometimes more than a hundred, can be associated with a particular plant species [1, 20, 23]. Some fungal endophytes maintain mutualistic associations with their hosts. For example, seed transmitted *Epichloë* species systemically infect several species of grasses, producing alkaloids which make the plants more resistant to herbivores [22]. Other endophytic species are known to improve disease resistance or abiotic stress tolerance in their hosts [25, 31]. However, for most endophytic species the effects of the symbiotic association on their hosts are unknown.

The associations between fungal viruses and their hosts are similar to plant-endophyte associations. Unlike plant or animal viruses, which are commonly associated with disease, many of the known fungal viruses cause no obvious symptoms [6, 9]. Only a few mycoviruses are known to affect their hosts, causing hypovirulence, disease [5, 18], or being beneficial. In fact, a mutualistic association between mycoviruses, endophytes, and their plant hosts, resulting in increased plant thermal tolerance, has been described recently [14]. Mycoviruses have not received as much attention as animal or plant viruses, but numerous fungal viruses, have been described since the first report of such a mycovirus was made by Hollings in 1962 [11]. Many of these viruses have double-stranded RNA (dsRNA) genomes, but species having ssRNA, and dsDNA genomes also exist [6, 9]. The purpose of the present investigation was to determine if mycoviruses were common in a collection of different species of endophytic fungi of grasses.

The fungal material analyzed consisted of 103 fungal isolates belonging to 53 species chosen randomly from a collection of endophytic fungi isolated from the grasses *Ammophila arenaria*, *Alopecurus arundinaceus*, *Brachypodium sylvaticum*, *Cynodon dactylon*, *Dactylis glomerata*, *Elymus farctus*, *Festuca rubra*, *Holcus lanatus*, and *Lolium perenne*. In endophyte surveys it is common for some species to be abundant, and for others to be represented by a single isolate [1, 20, 21]. Because of this, in our collection some species were represented by a single isolate. The species from which we purposely examined more isolates were *Beauveria bassiana* and *Torrubiella confragos*a, two entomopathogenic fungi which have often been isolated as endophytes from grasses and other plant species [2, 20, 21].

The screening for mycoviruses was based on the detection of dsRNA elements. This method allows the detection of genomes of dsRNA viruses, and of replicative forms of single-stranded RNA viruses [16]. Fungal isolates were cultured for 15-20 days on cellophane disks layered on top of potato dextrose agar in Petri plates. Approximately one gram of fresh mycelium was ground with liquid nitrogen, and dsRNA was extracted by CF-11 cellulose chromatography [16]. The purified dsRNA was treated with DNase I (Ambion TURBO DNA free), subjected to gel electrophoresis and the dsRNA elements visualized after staining with ethidium bromide.

DsRNA elements were detected in isolates belonging to 12 of the 53 endophytic species analyzed (Table 1; Fig. 1). The size of the dsRNA elements detected following electrophoresis of CF-11 cellulose extracts ranged from ca. 6.5 to 1.0 kbp (Table 1). Some infected isolates contained only one element, while others contained as many as four. The size of known mycovirus-associated dsRNA elements range from 13 kbp observed in the replicative forms of some members of the *Hypoviridae,* a family of single-stranded (+) RNA mycoviruses [17, 32], to 1.4 kbp in the bipartite genomes of the *Partitiviridae,* a family whose members have dsRNA genomes [7]. Therefore, the size, as well as the number of most observed dsRNA fragments suggested that they may represent mycovirus genomes.

Although partial or complete sequences of the RNA-dependent RNA polymerase (RdRp), or the coat protein gene are required to assign individual elements to mycovirus families, some characteristics of the dsRNA elements we observed allow us to speculate on the identity of some of the mycoviruses present in infected isolates. For example, several endophytic species harboured dsRNA molecules of a similar size to those of a totivirus genome, but also to the replicative form of some still unclassified ssRNA mycoviruses [12, 27, 29]. The *Totiviridae* family comprises viruses with dsRNA genomes consisting of a single linear molecule of 4.6 to 6.7 kbp [26]. This could be the case for some *Beauveria bassiana* and *Torrubiella confragosa* isolates carrying a 6.0 kbp dsRNA, *Mastigobasidium intermedium* (5.7 kbp), *Rhizoctonia bataticola* (6.5 kbp), *Tolypocladium cylindrosporum* (5.1 kbp), and *Valsa sp.* (4.5 kbp) (Table 1; Fig. 1). In *M. intermedium* and *T. cylindrosporum* the putative totivirus genomes, or ssRNA virus replicative forms of 5 – 6 kbp were accompanied by smaller dsRNA elements. This could indicate mixed infections by two or more viruses. Mixed infections by different mycoviruses, including totiviruses, have been reported in several fungal species [9], including *Epichloë festucae*, a grass endophyte [19]. In the case of *T. cylindrosporum*,

the minor dsRNA bands could correspond to the genome of a member of the family *Chrysoviridae*, whose genomes are composed of 4 segments of 2.4 to 3.6 kbp [8]. On the other hand, these small dsRNA fragments that accompany the ca. 5 kb dsRNAs, could also be replicative forms of ssRNA viruses [30], satellite RNAs, or defective derivatives of replication [28, 32]. An unidentified *Penicillium* species, *Curvularia inequalis*, and *Fusarium culmorum* also had dsRNA elements of size similar to that of the *Chrysoviridae*. Other species showed dsRNA patterns with characteristics of the family *Partitiviridae*, whose members have genomes composed of two dsRNA segments of 1.4 to 2.2 kbp [7]. This is the case for some of the *Beauveria bassiana* and *Torrubiella confragosa* isolates. The dsRNA patterns observed in these two clavicipitaceous entomopathogenic endophytes were very similar (Fig. 1B). In endophytic *Beauveria bassiana* 10 out of 15 isolates which were analyzed contained dsRNA. Two different dsRNA banding patterns were observed in this species, half of the infected isolates harboured one dsRNA element ca. 6.0 kbp in size, while the other half had two dsRNAs ca. 1.9 and 2.3 kbp in size (Fig. 1B). The incidence of virus infection in the endophytic isolates of this species was 67 %, greater than the 17 % observed in isolates obtained from soil in other studies [15]. In *Torrubiella confragosa* 3 out of 7 isolates were infected. The banding patterns in this species were very similar to those observed in *Beauveria bassiana*, with one type of infection comprising a single dsRNA element ca. 6.0 kbp in size, and the other type comprising two bands ca.1.9 and 2.3 kbp in size.

The occurrence of dsRNA elements and viruses has been previously reported in some of the fungal genera analyzed in this study including *Beauveria bassiana* where dsRNA elements, ca. 2 kbp in size, similar to the ones we describe here have been observed [4, 15]. Also a mycovirus with a similar dsRNA banding pattern to the one found in *Fusarium culmorum* here has been reported for *F. graminearum* [3] and some of the numerous viruses described in *Gaeumannomyces graminis*, have genomic dsRNA elements similar to the ones we show here [13]. Also, a 6.4 kbp dsRNA associated to hypovirulence occurs in some isolates of *Rhizoctonia solani* [24] In contrast *Curvularia* thermal tolerance virus has a genome consisting of two segments ca. 2.1 and 1.9 kbp in size [14], different from the larger dsRNAs we observed in *Curvularia inaequalis* (Fig. 1, Table 1) and while dsRNAs associated with hypovirulence have been described in *Valsa sp.* [10] to our knowledge the presence of virus-like dsRNAs had not been reported previously in any species of *Mastigobasidium*, *Tolypocladium*, or *Torrubiella.*

The sizes and banding patterns of the dsRNAs found in the various endophytic fungi suggests that mycoviruses from several known families are represented in our cohort, but until some molecular characterisation is available they cannot be assigned to any specific family.

Twenty-three percent of the 53 species analyzed contained virus-like dsRNA elements. Furthermore, this incidence of mycoviral infection among species could be an underestimation because several species that we analyzed were represented by a single isolate. Due to the apparently asymptomatic nature of their infection endophytic fungi were almost unknown until twenty years ago, but now they are considered ubiquitous organisms [9]. A similar situation exists with fungal viruses, and since the first mycovirus was observed [11] many more have been described [9]. In conclusion, the results of this survey suggest that mycoviral infections are relatively common among species of endophytic fungi of grasses.

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Table 1. Endophyte taxa where mycovirus-like dsRNA elements were detected. The host grass from where the fungus was isolated is shown in parentheses. Hosts from where endophytes were obtained were A: *Ammophila arenaria*, Al: *Alopecurus arundinaceus*, B: *Brachypodium sylvaticum*, C: *Cynodon dactylon*, D: *Dactylis glomerata*, E: *Elymus farctus*, F: *Festuca rubra*, H: *Holcus lanatus*, L: *Lolium perenne*, P: *Poa* sp**.** Size estimates were determined using agarose gel electrophoresis and dsDNA size markers.

Isolates infected / total analyzed	Host taxa	DsRNA elements observed*	
		Number	Size (kbp)
5/15	*Beauveria bassiana* (D, A, E, H)	1	6.0
5/15	*Beauveria bassiana* (D, A, E, H)	2	1.9 – 2.0
1/1	*Curvularia inaequalis* (A)	2	4.5 – 3.4
2/2	*Drechslera biseptata* (D)	2	1.0 – 1.5
1/3	*Fusarium culmorum* (D, F)	2	3 – 4.4
2/2	*Gaeumannomyces graminis* (H)	1	2.6
1/1	*Mastigobasidium intermedium* (H)	2	1.4 – 5.7
1/2	*Penicillium canescens* (D)	2	1.6 – 1.8
1/2	*Penicillium* sp. (P, C)	3	3.5 – 3.8 – 4.5
1/1	*Rhizoctonia bataticola* (A)	1	6.9
2/2	*Tolypocladium cylindrosporu*m (F, H)	4	3.4 – 3.7 – 4.2 – 5.1
2/7	*Torrubiella confragosa* (A, Al, B, D, E, L)	2	2 - 2.3
1/7	*Torrubiella confragosa* (A, Al, B, D, E, L)	1	6.0
1/1	*Valsa* sp. (D)	1	4.5

* No dsRNA was detected in any of the following endophytic species: *Acremonium strictum* (5 isolates analyzed), *Alternaria tenuissima* (1), *Ascochyta* sp. (1), *Aureobasidium pullulans* (1), *Botryosphaeria* sp. (1), *Chaetomium funicola* (1), *Chaetomium* sp. (4), *Cochliobolus sativus* (1), *Colletotrichum* sp. (1), *Coniothyrium cereale* (2), *Coprinellus radians* (1), *Cordyceps sinensis* (1), *Cryptococcus victoriae* (1), *Diaporthe viticola* (1), *Discula quercina* (1), *Drechslera dactylidis* (2), *Drechslera* sp. (3), *Fusarium oxysporum* (2), *Fusarium sporotrichoides* (1), *Gaeumannomyces cylindrosporus* (1), *Glomerella graminicola* (1), *Helgardia anguioides* (5), *Hypoxylon fuscum* (1), *Hypoxylon* sp. (1), *Lachnum* sp. (1), *Leptodontidium orchidicola* (3), *Leptosphaeria* sp. (1), *Mortierella* sp. (1), *Paecilomyces lilacinus* (1), *Penicillium janthinellum* (1), *Petriella guttulata* (1), *Phaeosphaeria nodorum* (1), *Phaeosphaeria* sp. (2), *Phoma herbarum* (1), *Plectosphaerella cucumerina* (1), *Podospora* sp. (3), *Pyrenochaeta* sp. (1), *Sporidiobolus* sp. (1), *Stemphylium solan*i (2), *Stilbella* sp. (2), *Volutella* sp. (1).

Fig. 1. Electrophoretic banding patterns of dsRNA elements isolated from *Tolypocladium cylindrosporum,* lane 1; *Fusarium culmorum,* 2; *Mastygobasidium intermedium,* 3; *Curvularia inaequalis,* 4; *Penicillium* sp. 5; *Gaeumannomyces graminis* 6; *Rhizoctonia bataticola,* 7. **B**. Two different dsRNA patterns were observed in different isolates of *Beauveriabassiana,* 8, 9, and *Torrubiella confragosa*, 10, 11. Lanes M contain λ-*Hin*d III size marker, numbers on left indicate kbp.