Viruses infecting macrofungi

Ergin Sahin¹ · Ilgaz Akata¹

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Abstract Ever since their discovery just about 56 years ago in the cultivated mushroom *Agaricus bisporus*, many more viruses infecting fungi have been identified in a wide range of fungal taxa. With mostly being asymptomatic, especially the ones that are detrimental to their phytopathogenic hosts are intensively studied due to their considerable importance in developing novel plant protection measures. Contrary to the rapid accumulation of notable data on viruses of plant pathogenic microfungi, much less information have hitherto been obtained in regards to the viruses whose hosts are macrofungi. According to the current literature, only more than 80 distinct viruses bearing either linear dsRNA or linear positive sense ssRNA genome and infecting a total number of 34 macrofungal species represented with four *Ascomycota* and 30 *Basidiomycota* have been identified so far. Among these 34 macrofungal species, 14 are cultivated edible and wild edible mushroom species. According to the 10th ICTV (International Committee on Taxonomy of Viruses) Report, macrofungal viruses with linear dsRNA genome are classified into five families (*Partitiviridae*, *Totiviridae*, *Chrysoviridae*, *Endornaviridae* and *Hypoviridae*) and macrofungal viruses with linear positive sense ssRNA genome are classified into seven families (*Betaflexiviridae*, *Gammaflexiviridae*, *Barnaviridae*, *Narnaviridae*, *Virgaviridae*, *Benyviridae* and *Tymoviridae*). In this review, following a brief overview of some general characteristics of fungal viruses, an up to date knowledge on viruses infecting macrofungal hosts were presented by

summarizing the previous, recent and prospective studies of the field.

Keywords Fungal virus · Macrofungi · Viral diversity · Infection

Introduction

Viruses are intracellular parasitic microorganisms that exploit the host cell metabolism in favor of their replication. The term “Fungal virus” (or mycovirus) is used to refer to the particular viruses that specifically infect fungi. Macrofungi are specific part of the kingdom fungi whose sporophores are visible with naked eye and they comprise two phyla: *Ascomycota* and *Basidiomycota* with large, easily observable spore-bearing fruit bodies [75]. It was reported that many fungal species including macrofungi are ubiquitously infected with members of more than one different virus families together [2–97].

Discovery of fungal viruses were made relatively late compare to the identification and characterization of viruses of animals, plants and prokaryotes [28, 29]. This is perhaps mostly due to the cryptic or asymptomatic nature of mycoviruses [57]. Since the first identification of viruses which give rise to Dieback (or La France) disease in cultivated mushroom *Agaricus bisporus* (J.E. Lange) Imbach, our knowledge of fungal viruses has increased gradually over the past 56 years [17]. However, from the stand point of macrofungal virology, early studies of fungal viruses infecting macrofungi were almost limited to their demonstration using electron microscopy and did not exceed far beyond the determination of their nucleic acid type and their serological features. In fact, despite their high diversity, much less is known about macrofungal viruses compare to the viruses of other fungi.

✉ Ergin Sahin
erginsahin@ankara.edu.tr

¹ Faculty of Science, Department of Biology, Ankara University, Ankara, Turkey

Just as viruses that infect animals, plants and prokaryotes, fungal viruses need the living cells of a fungus to propagate. However, fungal viruses have the following unique characteristics that are not present in viruses of other organisms: Firstly, the life cycles of almost all of the fungal viruses do not involve any extracellular route for infection. Therefore entry of fungal viruses into fungus host cell is thought to be a quite uncommon and earlier incident. Secondly, Fungal viruses are transmitted from one individual to another only in an intracellular fashion including vertical transmission mediated by sexual or asexual spores and horizontal transmission through cell-to-cell fusion which is also known as hyphal anastomosis [18]. Lastly, Fungal viruses are lack of the ability for self locomotion within their host. In other words, the cell to cell movement proteins which are crucial for the life cycles of animal and plant viruses are absent in the fungal viruses. There are some cellular evidences suggesting that fungal viruses move forward by cytoplasmic streaming between the host cells [72].

In regards to the host ranges of fungal viruses, Due to the restrictive effects of vegetative incompatibility on the reproduction of fungi, the natural host ranges of fungal viruses are limited to individuals found in the same or closely related vegetative compatibility groups [55]. However, recently, fungal viruses that have naturally broadened host ranges have been identified. For instance, direct and indirect evidences obtained from in vitro infection assays and natural environment respectively showed that some viruses of the genus *Heterobasidion* can be transmitted from one *Heterobasidion* species to another [90]. Fungal virus transmission between distantly related fungal species is considered to be an extremely rare event [36]. Nevertheless, there is evolutionary evidences that Partitiviruses have been exchanged between plants and fungi [70, 52]. In fact, recent studies on the ecology of fungal viruses revealed new evidences that fungal viruses may have wider host ranges than generally thought. In a recent study mainly conducted to evaluate whether closely related fungal viruses could inhabit distant fungal species in a single habitat, it was revealed that while not so often, conspecific viruses are transmitted between distantly related fungal species via a mechanism that has yet to be explained [95].

Considering the evolution of fungal viruses, two fundamental hypothesis named as “ancient coevolution hypothesis” and “plant virus hypothesis” respectively have been proposed to elucidate the origin of fungal viruses [57]. In the “ancient coevolution hypothesis” it is claimed that eventhough the origin of fungal viruses is still uncertain, the relationship between fungal viruses and fungi is ancient and represents a long-term coevolutionary process. In contrast with this, in the “plant virus hypothesis,” it is

suggested that fungal viruses derived relatively lately from plant viruses. Accordingly, the actual fungal virus was once a plant infecting virus that eventually translocated from plant to fungus live on the same host plant. Since there is currently no reliable data regarding the evolution of fungal viruses, their origin still remains unknown.

In regards to their impact on host phenotype, although the phenotypic effects of mycoviral infections can vary from beneficial to harmful, most of them are asymptomatic or cryptic [19]. Thus, in general, fungal viruses do not appear to disturb their hosts’ fitness. This circumstance indicates the well adaptation of fungal viruses to the coexistence with their hosts for long period of time. Seeming as asymptomatic at first, may not always mean fungal viruses are unnoticed by their hosts. Indeed, symptoms may only appear when certain conditions (environmental or cellular) of the virus-fungus interaction are altered [12]. Yet the viral and host factors that regulate the phenotypic outcomes of a given fungal virus—fungus-combination are still unknown and need to be addressed. This is perhaps a quite challenging topic for mycovirologists due to the absence of suitable transfection or infectivity assays which hamper confirming Koch’s postulates and mostly prevent the researcher from getting a rational conclusion. Furthermore, as mostly being infected with unknown numbers of viruses, it is very troublesome to link a certain phenotypic abnormality of fungi with the investigated virus. Studies carried out more than a decade to unveil the causative agents of La France disease and Mushroom Virus X disease in *Agaricus bisporus* or the dieback disease in oyster mushroom *Pleurotus ostreatus* can be given as solid examples to illustrate this difficulty.

Most known viruses infecting macrofungi have dsRNA genomes enclosed in a capsid with icosahedral symmetry, yet a growing number of positive-sense ssRNA viruses with macrofungal origin (constituting about 30% of the known macrofungal viruses) have also been discovered and characterized. The list of currently known fungal viruses that infect macrofungi and their phenotypic effects on their hosts were summarized in Tables 1 and 2. So far, negative sense ssRNA viruses and any kind of DNA viruses have never been discovered in macrofungi. The absence of DNA viruses in macrofungal species may be explained to a certain extent by the phenomenon called Repeat Induced Point Mutation (RIP) [13, 24]. RIP is a premeiotic mechanism present in fungi which regulates the elimination of duplicated or repeated DNA sequences with sizes more than around 400 bp in the genome. Thus it could be possible that RIP might prevent the replication of DNA viruses in the fungal species. However there is currently no experimental settings to address this hypothesis.

According to the 10th ICTV (International Committee on Taxonomy of Viruses) Report, macrofungal viruses

Table 1 List of dsRNA viruses that infect macrofungi

Genome diversity	Family/genus	Virus	Host	Disease	References
Linear dsRNA	<i>Partitiviridae</i>	Heterobasidion Partitivirus 1 (HetPV1)	<i>Heterobasidion abietinum</i>	Conditional	[90]
		Heterobasidion Partitivirus 2 (HetPV2)	<i>Heterobasidion parviporum</i>		[97, 93]
		Heterobasidion Partitivirus 3 (HetPV3)	<i>Heterobasidion ecrustosum</i>		[94]
		Heterobasidion Partitivirus 4 (HetPV4)	<i>Heterobasidion parviporum</i>		[90, 96]
		Heterobasidion Partitivirus 5 (HetPV5)	<i>Heterobasidion parviporum</i>		[90]
		Heterobasidion Partitivirus 7 (HetPV7)	<i>Heterobasidion parviporum</i>		[97]
		Heterobasidion Partitivirus 8 (HetPV8)	<i>Heterobasidion irregulare</i> Garbel. & Otrosina		[89]
		Heterobasidion Partitivirus 9 (HetPV9)	<i>Heterobasidion parviporum</i>		[97]
		Heterobasidion Partitivirus 10 (HetPV10)	<i>Heterobasidion occidentale</i>		[90]
		Heterobasidion Partitivirus 11 (HetPV11)	<i>Heterobasidion parviporum</i> <i>Heterobasidion annosum</i> <i>Heterobasidion australe</i> Y.C. Dai & Korhonen		[90, 91]
		Heterobasidion Partitivirus 12 (HetPV12)	<i>Heterobasidion annosum</i>		[38]
		Heterobasidion Partitivirus 13 (HetPV13)	<i>Heterobasidion annosum</i> <i>Heterobasidion parviporum</i>		[38]
		Heterobasidion Partitivirus 14 (HetPV14)	<i>Heterobasidion irregulare</i>		[38]
		Heterobasidion Partitivirus 15 (HetPV15)	<i>Heterobasidion parviporum</i>		[38]
		Heterobasidion Partitivirus 17 (HetPV17)	<i>Heterobasidion irregulare</i>		Unpublished
		H. annosum P-type partitivirus(HetPVP)	<i>Heterobasidion annosum</i>		[34]
		A. bisporus virus 4	<i>Agaricus bisporus</i>	Symptomatic	[3]
		P. ostreatus virus 1 (PoV1)	<i>Pleurotus ostreatus</i>	Asymptomatic	[45]
		P. ostreatus virus Shin-Nong (PoV-SN)	<i>Pleurotus ostreatus</i>	Asymptomatic	[39]
		Oyster mushroom isometric virus 1 (OMIV-1)	<i>Pleurotus ostreatus</i>	Dieback disease	[104]
		Oyster mushroom isometric virus 2 (OMIV-2)	<i>Pleurotus ostreatus</i>	Dieback disease	[104]
		Oyster mushroom isometric virus (OMIV)	<i>Pleurotus ostreatus</i>	Dieback disease	[65]
		F. velutipes browning virus (FvBV)	<i>Flammulina velutipes</i>	Brown discoloration	[48]
		F. velutipes isometric virus (FvIV)	<i>Flammulina velutipes</i>	Symptomatic	[48]
		C. purpureum cryptic virus 1	<i>Chondrostereum purpureum</i>	Asymptomatic	[77]
		L. edodes partitivirus 1 (LePV1)	<i>Lentinula edodes</i>	Conditional	[23]
		M. platyphylla Virus	<i>Megacollybia platyphylla</i>	Unknown	[95]
		L. tabidus RNA Virus 1 (LrRV1)	<i>Lactarius tabidus</i>		
		L. rufus RNA Virus 1 (LrRV1)	<i>Lactarius rufus</i>		

Table 1 continued

Genome diversity	Family/genus	Virus	Host	Disease	References
	<i>Totiviridae</i>	T. aestivum virus 1 (TaV1)	<i>Tuber aestivum</i>	Asymptomatic	[85]
	<i>Chrysoviridae</i>	LaFrance isometric virus, (LFIV)/A. bisporus virus 1 (AbV1)	<i>Agaricus bisporus</i>	La France Disease	[44, 21]
	<i>Endornaviridae</i>	A. bisporus Endornavirus 1 (AbEV1)	<i>Agaricus bisporus</i>	Mushroom virus × Disease	[46]
		T. aestivum endornavirus (TaEV)	<i>Tuber aestivum</i>	Asymptomatic	[83]
	<i>Hypoviridae</i>	A. bisporus Virus 2 (AbV2)	<i>Agaricus bisporus</i>	Unknown	[9]
		A. bisporus Virus 10 (AbV10)			
		A. bisporus Virus 11 (AbV11)			
	Unclassified	L. edodes mycovirus HKB (LeVHKB)	<i>Lentinula edodes</i>	Asymptomatic	[49]
		L. edodes mycovirus HKA (LeVHKA)	<i>Lentinula edodes</i>		[53]
		L. edodes Spherical Virus (LeSV)	<i>Lentinula edodes</i>	Unknown	[100]
		L. edodes Virus	<i>Lentinula edodes</i>	Asymptomatic	[87]
		T. terrestris Virus 1 (TtV1)	<i>Thelephora terrestris</i>	Asymptomatic	[58]
		V. volvacea Virus (VvV)	<i>Volvariella volvacea</i>	Asymptomatic	[6, 7]
		C. odora virus 1 (CoV1)	<i>Clitocybe odora</i>	Small Cap or Morel Like Phenotype	[27]
		Heterobasidion RNA virus 6 (HetRV6)	<i>Heterobasidion annosum</i> <i>Heterobasidion parviporum</i> <i>Heterobasidion abietinum</i> <i>Heterobasidion occidentale</i>	Conditional	[91]
		P. sapidus Spherical Virus (PsSV), P. sapidus Rod Shaped Virus (PsRSV)	<i>Pleurotus cornucopiae</i> (Paulet) Rolland (Syn.: <i>Pleurotus sapidus</i> Sacc.)	Asymptomatic	[59]
		C. aegerita virus	<i>Cyclocybe aegerita</i>	Asymptomatic	[2]

with linear dsRNA genome are classified into five families. These are: (1) *Partitiviridae* family which is characterized with having two genomic segments that encode capsid protein and an RNA dependent RNA polymerase (RdRp) respectively and are encapsidated separately in a capsid with $T = 2$ icosahedral symmetry, (2) *Totiviridae* family whose members have capsid structure with $T = 2$ icosahedral symmetry and a monopartite genome that encodes for a capsid protein and an RdRp, (3) *Crysoviridae* family, the members of which have four genomic segments that are encapsidated separately in a capsid with $T = 1$ icosahedral symmetry and (4) *Endornaviridae* and (5) *Hypoviridae* families which are defined with bearing single genome segment encoding for RdRp with no true capsid.

On the other hand, macrofungal viruses with linear, positive-sense, ssRNA genome are classified into seven families according to the 10th Report of ICTV. These are: (1) the *Betaflexiviridae* and (2) *Gammaflexiviridae* families which are mainly characterized with flexuous and filamentous virion structures, (3) the *Virgaviridae* and (4) *Benyviridae* families whose members have rod shaped capsid structure displaying helical symmetry, (5) the *Barnaviridae* family which are identified with their

bacilliform virion structure with $T = 1$ icosahedral symmetry harboring a monopartite genome, (6) the *Narnaviridae* family, the members of which are mostly located in mitochondria, have no true virion and consist of naked monopartite genome associated with an RdRp and lastly (7) the *Tymoviridae* family whose members have monopartite genome which encodes a replication machinery including RdRp along with a capsid protein that composes a virion structure with $T = 3$ icosahedral symmetry.

Due to the lack of a comprehensive review study which specifically concentrated on viruses infecting macrofungi, we foresaw a necessity to prepare this review and thereby aimed to contribute to the current literature of the field. In the rest of this review study, we briefly presented the biology of viruses infecting macrofungi from both phylum *Ascomycota* and phylum *Basidiomycota*. To simply and comprehensibly present the literature, we organized this review part under three sections which are “viruses of wood decaying macrofungi”, “viruses of edible macrofungi” and “other macrofungal viruses”. The review was later ended with a concluding remarks section in which the prospective studies of the field are discussed.

Table 2 List of ssRNA viruses and viruses with unknown genome type that infect macrofungi

Genome diversity	Family/genus	Virus	Host	Disease	References
Linear +ssRNA	<i>Betaflexiviridae</i>	A. bisporus Virus 3 (AbV3), A. bisporus Virus 5 (AbV5), A. bisporus Virus 6 RNA1 (AbV6 RNA1)	<i>Agaricus bisporus</i>	Unknown	[9]
		A. bisporus Spherical virus (AbSV)	<i>Agaricus bisporus</i>	Unknown	Unpublished GenBank accession: AGH07918.1, 2013
	<i>Gammaflexiviridae</i>	A. bisporus Virus 7 (AbV7), Agaricus bisporus Virus 9 (AbV9)	<i>Agaricus bisporus</i>	Unknown	[9]
	<i>Barnaviridae</i>	Mushroom bacilliform virus (MBV)	<i>Agaricus bisporus</i>	Conditional	[63]
	<i>Narnaviridae</i>	T. excavatum mitovirus (TeMV)	<i>Tuber excavatum</i>	Asymptomatic	[82]
		T. aestivum mitovirus (TaMV)	<i>Tuber aestivum</i>	Asymptomatic	[84]
		Heterobasidion Mito Virus 1 (HetMV1)	<i>Heterobasidion annosum</i>	Conditional	[92]
		A. bisporus Mitovirus 1 (AbMV1), A. bisporus Virus 14 (AbV14), A. bisporus Virus 15 (AbV15)	<i>Agaricus bisporus</i>	Unknown	[9]
	<i>Virgaviridae</i>	A. bisporus Virus 6 RNA2 (AbV6 RNA2)	<i>Agaricus bisporus</i>	Symptomatic	[9]
	<i>Benyviridae</i>	A. bisporus Virus 8 (AbV8), A. bisporus Virus 13 (AbV13)	<i>Agaricus bisporus</i>	Unknown for both	[9]
	<i>Tymoviridae</i>	Oyster Mushroom Spherical Virus (OMSV)	<i>Pleurotus ostreatus</i>	Oyster Mushroom Die Back Disease	[104]
	Ambsetviridae (Newly Proposed Family)	A. bisporus Virus 12 (AbV12)	<i>Agaricus bisporus</i>	Unknown	[9]
		A. bisporus Virus 16 (AbV16)/Brown Cap Mushroom Virus (BCMV)	<i>Agaricus bisporus</i>	Brown cap mushroom disease	[9]
	Unclassified	L. Edodes ssRNA Virus	<i>Lentinula edodes</i>	Symptomatic	[103]
Undetermined	Unclassified	B. edulis Virus	<i>Boletus edulis</i>	Asymptomatic	[29]
		B. edulis Filamentous Virus	<i>Boletus edulis</i>	Inconclusive	[30]
		B. edulis Polyhedral Virus 1		Association with Little Cap Disease	
		B. edulis Polyhedral Virus 2			
		B. edulis Polyhedral Virus 3			
		L. laccata virus	<i>Laccaria laccata</i>	Microcephalic Sporophores	[4]
		L. amethystina virus	<i>Laccaria amethystina</i>		
		C. infundibuliformis Virus	<i>Craterellus tubaeformis</i> (Fr.) Quél. (Syn.: <i>Cantharellus infundibuliformis</i> (Scop.) Fr.)		
		C. lagopus virus	<i>Coprinopsis lagopus</i>	Asymptomatic	[76]
		A. multiforme virus	<i>Annulohyphoxylon multiforme</i>	Asymptomatic	[102]
		S. commune virus	<i>Schizophyllum commune</i>	Mycelial disintegration	[40]
		P. eryngii spherical virus (PeSV)	<i>Pleurotus eryngii</i>	Yes	[64]
		N. lepideus Virus	<i>Neolentinus lepideus</i> (Fr.) Redhead & Ginns	Asymptomatic	[51]
		G. peronatus Virus	<i>Gymnopus peronatus</i> (Bolton) Gray	Asymptomatic	
		P. aurea Virus	<i>Phaeolepiota aurea</i> (Matt.) Maire	Asymptomatic	
		P. ostracoderma virus(POV)	<i>Peziza ostracoderma</i>	Asymptomatic	[10]

Viruses of wood decaying macrofungi

Thus far, viruses of wood decaying macrofungi have been studied comprehensively in the genus *Heterobasidion* and less detailedly in the species *Chondrostereum purpureum* (Pers.) Pouzar, *Schizophyllum commune* Fr. and *Annulohyphoxylon multifforme* (Fr.) Y.M. Ju, J.D. Rogers & H.M. Hsieh [22–102].

Viruses of *Heterobasidion* spp

Probably, one of the macrofungal genera, the viruses of which are particularly being studied in detail, is the genus *Heterobasidion*. Viruses of *Heterobasidion* spp. are the only virus group among the macrofungal viruses that are investigated primarily for their potentials in being employed as biocontrol agents and for this reason they deserve a special interest. Members of the genus *Heterobasidion* constitute one of the most destructive conifer pathogens which are widespread mostly in the boreal forests and causes severe economic losses [101, 73]. Upon spore mediated infection of fresh stumps, transmission of *Heterobasidion* spp. takes place efficiently from an infected tree to a healthy one via mycelia growth occur on the roots of neighboring trees which contact each other [81]. Due to the inadequacy of available preventive measures including biological and chemical stump treatment against the spore infection and stump removal, utilization of fungal viruses as a prevention modality in keeping *Heterobasidion* mediated forest damages under control appears to be promising.

Approximately 15% of the *H. annosum* (Fr.) Bref. and *H. parviporum* Niemelä & Korhonen present in Europe and western Asia were found to host dsRNA viruses quite commonly with a state of coinfection [34]. The majority of the dsRNA viruses identified in *Heterobasidion* spp. belongs to the *partitiviridae* family and at least 13 different partitivirus species have been identified in *Heterobasidion* spp. thus far [97, 90, 29–89]. *Heterobasidion* partitiviruses are ubiquitously found in both pathogenic and saprothropic *Heterobasidion* species from different regions of the world including Europe, North America and Asia. However partitiviruses represent only less than 30% of the viral infections with dsRNA origin hosted by *Heterobasidion* spp [90]. The taxonomically unclassified virus species *Heterobasidion* RNA Virus 6 (HetRV6) is indeed the most commonly encountered dsRNA virus in the *Heterobasidion* spp. which constitutes more than 70% of the dsRNA virus infections in the genus *Heterobasidion* [91]. This virus appears to be distantly related to *Curvularia* Thermal Tolerance Virus (CThTV) [50]. HetRV6 is widely spread out in Europe and North America, however it has not been

found in East Asia. Compared to the partitiviruses, HetRV6 exhibits relatively less sequence diversity yet a great number of various HetRV6 strains can be observed even within a single forest terrain [97]. More recently, using next generation sequencing-based survey of small RNAs in *H. annosum*, a mitovirus from a *Narnaviridae* family denominated as *Heterobasidion* mitovirus 1 (HetMV1) was identified [92]. Despite the fact that HetMV1 has a little larger genome size compared to mitoviruses, its genome solely encodes for an RdRp as the typical characteristics of mitoviruses. The closest relative of HetMV1 was found to be *Clitocybe odora* Virus 1 which shares only about 33% amino acid sequence identity with HetMV1 [27]. Although mitoviruses are prevalent in fungi, only six mitoviruses have so far been identified in macrofungi (2 from ascomycota and 4 from basidiomycota) (Table 2). Whether mitoviruses are also hosted by other *Heterobasidion* species has not been revealed yet.

Both in vitro and field studies have shown that transmission of *Heterobasidion* viruses can occur interspecies as well as intraspecies manner within the genus *Heterobasidion* [97, 90, 94, 93, 91, 36–37]. This phenomenon looks quite unusual as vegetative incompatibility frequently prevents transfer of fungal viruses both in between different species and even within same species of a given fungal genus. For this reason, vegetative incompatibility still remains as one of the most important issue to be overcome by the researchers who especially works on developing mycovirus based biocontrol agents.

In vitro transmission studies of fungal viruses are conducted by using the dual method of mycelial culturing. Accordingly, mycelia of infected donor strain is paired on the same agar plate with the virus-free recipient strain. Later on, the mycelial colonies are allowed to meet. After then, samples collected from the recipient site are analyzed for virus infection along with performing genotype analysis to check whether the recipient culture is contaminated with the virus infected donor strain at any degree. The results of the infection studies carried out with *Heterobasidion* spp. revealed that *Heterobasidion* partitiviruses HetPV2 and HetPV9 can be transmitted from *H. parviporum* to *H. araucariae* [8].

Apart from the fact that there are indirect evidences of virus transmission in between different *Heterobasidion* species obtained from field studies [90, 91], the mechanisms of *Heterobasidion* virus transmission was also studied in their natural environments. Theoretically, horizontal transmission of *Heterobasidion* viruses may involve mycelial mating between two homokaryon or a homokaryon and a heterokaryon or contact between two somatically incompatible heterokaryotic cells [94, 32]. In a 2 years long field study, intraspecies transmission of HetPV4 was investigated [96]. In this study, HetPV4

infected mycelia of *H. parviporum* were inoculated onto stumps that are naturally infected with *H. parviporum* and after 2 years of inoculation, HetPV4 infection was observed in the resident *H. parviporum* strains settled on stumps and root systems. Genotyping of microsatellites of these *H. parviporum* strains before and after HetPV4 infection demonstrated that intraspecies horizontal transmission of HetPV4 appears to occur with two different fashions which are (1) mycelial anastomosis between extraneous, infected heterokaryotic donor and indigenous homokaryotic recipient with different genotype and (2) contact between two heterokaryotic mycelia which are somatically incompatible [96].

Considering long distance, spore mediated, vertical dispersion of *Heterobasidion* viruses, the presence of dsRNA was demonstrated in basidiospore-generated cultures in vitro [33]. Additionally, in a field study carried out in a *Heterobasidion* infected forest site of Southern Finland, HetRV6 dsRNAs, but not that of the others, were detected in air carried basidiospores of *Heterobasidion* [97]. If *Heterobasidion* partitiviruses and HetMV1 are actually lack of this transmission route, then this might be an explanation for their relatively lower prevalence compare to HetRV6. Besides, whether secondary vectors such as insects and nematodes play a crucial role in the short-range dispersion of *Heterobasidion* viruses remains to be elucidated.

Another remarkable finding regarding *Heterobasidion* viruses is that they tend to accumulate in the aging infection centers of *Heterobasidion* spp [97]. In a 7 years of intensive field study primarily carried out to investigate spatial and temporal changes in the communities of *Heterobasidion* viruses that are hosted by *H. parviporum*, viruses were found to be accumulated over time in clonal host strains due to the spread of resident viruses within and between indigenous *H. parviporum* strains in addition to the different, extraneous virus strains arriving via airborne spores. About 48% of the resident *Heterobasidion* strains were found to be infected which was quite higher than the total viral incidence observed in the randomly collected *Heterobasidion* isolates (15%) [9]. Another noteworthy finding of this study was that coinfections by distant *Heterobasidion* viruses are more likely to persist than those closely related strains in a given viral community which suggests an involvement of mutual exclusion in this condition.

Regarding the effects of *Heterobasidion* viruses on the phenotypic characteristics of their hosts, preliminary studies showed that mycoviruses seem to have no discernible effects on *Heterobasidion* spp [90, 28–93, 91]. However in a more recent and comprehensive study carried out for examining the effects of *Heterobasidion* viruses on their hosts, two different viruses, HetRV3 and HetRV6,

isolated from *H. ecrustosum* Tokuda, T. Hatt. & Y.C. Dai and *H. abietinum* Niemelä & Korhonen respectively were transmitted to other *Heterobasidion* species in vitro [31]. The experiment was performed with isogenic virus-free and virus-infected *Heterobasidion* isolates in order to examine the effects of these two viruses on the host growth rate. As a result, it was shown that each virus strain has distinctive effects on different *Heterobasidion* host strain and furthermore depending on the environmental and ecological conditions, a single virus strain sometimes have variable effects on the fitness of a single host isolate (varying from harmful to beneficial) [31]. Besides, in another study, HetPV1, HetPV2 and HetPV3 whose natural host species are *H. abietinum*, *H. parviporum* and *H. ecrustosum* respectively were shown to exhibit more obvious phenotypic effects upon host switching [37]. In this study, HetPV1, HetPV2 and HetPV3 were transferred to the foreign host species *H. occidentale* Otrösina & Garbel which has no common evolutionary background with the transferred viruses and is distantly related to the natural host species of these viruses. As a result, all viruses significantly affected the growth rate of the *H. occidentale* in a temperature dependent manner with mostly being negative except for HetPV2 which also had promotive effect at a certain temperature [37].

With respect to the gene expression features of *Heterobasidion* viruses, as the characteristic of partitiviruses, in *Heterobasidion* partitiviruses, generally the capsid protein (CP) encoding genome segment is more abundantly produced compared to the RdRp genome segment in infected mycelium [37]. This differential production is also observed at the transcript level. In *Heterobasidion* partitiviruses, the ratios of the genome segments were found to be unique to each studied virus and these ratios were also shown to be generally constant in different hosts and at different temperatures [37]. In one of the most extreme case, tenfold higher CP segment quantity than the quantity of RdRp segment was observed in HetPV12 infected *H. annosum* strain which give rise to thought that the distribution of these two particle types would be in some way regulated in the infected mycelia [37]. On the other hand there are some exceptions among *Heterobasidion* partitiviruses in which the CP/RdRp ratios are significantly lower. In the case of HetRV3 and HetRV13, the quantities of RdRp segments were found to be much higher than the CP segment quantities in the infected hosts [92, 37]. This observation along with the findings that these two viruses also cause the most harmful effects on their hosts would suggest that the excessive production of RdRp would have something to do with these phenotypic conditions perhaps via interfering with the host RNA pool [37, 31].

In other respects, *Heterobasidion* spp. appear to possess all the cellular components that operate the RNA

interference (RNAi) mechanism [92, 54]. There are well documented evidences that RNA silencing is widely used by *Heterobasidion* spp. as a mechanism to protect against fungal viruses. In a small RNA deep sequencing study conducted on various *Heterobasidion* strains, siRNA molecules were found to be produced from all nine *Heterobasidion* virus genomes present in the studied isolates including the belatedly identified mitovirus HetMV1 [92].

In a more recent study primarily carried out to assess the safeness of using *Heterobasidion* viruses as the biocontrol agents, interspecies transmission of *Heterobasidion* viruses were investigated in different mycorrhizal and saprotrophic fungal species inhabiting at a single forest site where the spatial distributions of *Heterobasidion* viruses in *H. parviporum* clonal individuals were previously mapped [95]. The presence of *Heterobasidion* viruses in mycelia samples collected from fruit bodies, in vitro cultured isolates and in-growth mesh bags were examined with RT-PCR analyses. As a result only 3% of the fruit bodies and 2% of the mycorrhizal samples grown in mesh bags but none of the in vitro cultured isolates were demonstrated to harbor *Heterobasidion* viruses. Besides, a strain of HetPV9 which shares 94% RdRp sequence similarity with the HetPV9, originally isolated from *Heterobasidion parviporum*, was defined in the saprotrophic macrofungi *Megacollybia platyphylla* (Pers.) Kotl. & Pouzar. This finding also supports the concept that conspecific virus pools can be shared between evolutionarily distant fungal species. Although it appears to be an occasional event, observation of conspecific virus infections within distantly related fungal species suggested that the highly infected individuals of a particular fungal species could lead to a “pathogen spillover” which results in transmission of viruses even to the far-related species [60]. *Heterobasidion* virus transmission from an infected *H. parviporum* strain to the distantly related species could not be established using in vitro dual culturing method which indicated that hyphal interaction does not take part in this way of virus transmission [95]. Currently, the routes of interspecies fungal virus transmission remain unknown and need to be addressed. Since, owing to the presence of differences in virus tolerance, an artificially brought on interspecies fungal virus transmission in nature might have a major ecological influence on fungal communities.

Viruses of *C. purpureum*, *S. commune* and *A. multifforme*

With the purpose of finding out the factors that may influence the virulence of *Chondrostereum purpureum*, which is used as a mycoherbicide for the control of a wide variety of deciduous trees, 20 isolates of *C. purpureum* were examined for the presence of dsRNA elements [77].

As a result, only one isolate exhibiting no abnormality was revealed to harbor two linear dsRNA molecules associated with a 30 nm virus particle. Therefore, the virus was named as *Chondrostereum purpureum* Cryptic Virus (CPCV). CPCV dsRNAs, roughly 1.9 and 1.7 kbp in length, were designated as dsRNA1 and dsRNA2 respectively and subsequently sequenced. Bioinformatical analysis of these two genome segments revealed the dsRNA1 to encode a putative 587 amino acid long RdRp with a predicted molecular weight of 68.1 kDa and the dsRNA2 to encode a deduced 52.8 kDa capsid protein (CP) comprised of 480 amino acid. Phylogenetic analysis based on the amino acid sequences of both CPCV RdRp and CP associated this virus to the family *Partitiviridae* [77]. The possible effect of CPCV on *C. purpureum* has yet to be addressed and revealed in a conclusive manner.

A spherical, 130 nm virus leading to mycelial disintegration was identified in *Schizophyllum commune* using electron microscopy. The frequency of the mycelial disintegration was correlated with the abundance of virus particles present in the cytoplasm of mycelia using both disease sensitive and resistant strains [40].

Lastly, an icosahedral virus with a diameter of approximately 32 nm was identified in a healthy strain of *Annulohyphoxylon multifforme* using electron microscopy yet have not been further characterized [102].

Viruses of edible macrofungi

Up until now, the presence of fungal viruses have only been demonstrated in a total number of 14 different edible macrofungal species comprising of seven cultivated edible and seven wild edible mushroom species (Tables 1, 2). These are *Agaricus bisporus* (J.E. Lange) Imbach, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Flammulina velutipes* (Curtis) Singer, *Lentiluna edodes* (Berk.) Pegler, *Cyclocybe aegerita* (V. Brig.) Vizzini (Syn.: *Agrocybe aegerita* (V. Brig.) Singer), *Boletus edulis* Bull. and *Volvariella volvacea* (Bull.) Singer for the cultivated edible macrofungi and *Tuber aestivum* Vittad., *Tuber excavatum* Vittad., *Laccaria laccata* (Scop.) Cooke, *Laccaria amethystina* Cooke, *Craterellus tubaeformis* (Fr.) Quél. (Syn.: *Cantharellus infundibuliformis* (Scop.) Fr.), *Lactarius rufus* (Scop.) Fr. and *Lactarius tabidus* Fr. for the wild edible macrofungi. Among the viruses that infect edible macrofungi, most particularly the ones that generate disease on cultivated edible macrofungi are of great importance because of leading to considerable economic losses in mushroom industry. As being in the forefront of the commercial mushroom cultivation, it is not surprising that *Agaricus bisporus* is the first fungus species in which fungal viruses were discovered.

Viruses of *Agaricus bisporus*

Up until now, two distinct viral diseases named as La France disease and Mushroom Virus X disease have been identified in *Agaricus bisporus*. The first reported outbreak of the La France disease took place in 1948 on the farms of La France brothers in Pennsylvania, USA [78] and few years later, similar symptoms associated with this disease were also observed in various European countries and in Australia [16]. At that time, a number of different denotations including “dieback”, “watery stipe”, “X-disease”, “Mummy” and “brown disease” were used to refer La France disease [105]. La France disease occurred with various degrees of epidemics in North America and Europe and became a primary problem in the mushroom industry during 1970s [68]. The first sign of La France disease is the retarded growth of fruiting bodies and as a consequence, the formation of open areas all across the cultivation beds resulting in yield loss. Diseased sporophores encompassing the bare fields exhibits a typical phenotype consisting of swollen stipes and small diminished caps. Dark brown colored caps and twisted stipes are also occasionally observed as the symptoms of the disease. Infected spores are released from the diseased mushrooms which usually mature untimely [68]. Heat treatment (33 °C) of infected young mycelia for few weeks was shown to eradicate fungal viruses or at least reduce virus titres and used as a preventive measure to a certain extent [11]. The infectious aspects of La France disease was revealed ahead of the demonstration of its causative agents as viruses [74]. The viruses causing La France disease can be transmitted both vertically by infected basidiospores which comprise about 65–75% of the spores produced and horizontally from infected mycelia to healthy one via hyphal fusion [49–80].

As the result of intensive and careful investigations carried out for revealing the causal agents of La France disease, nine dsRNA elements was found to be fundamentally associated with the disease [25, 41]. The dsRNA molecules prevail among mushrooms with La France disease include six major dsRNAs: L1 (3.8 kbp), L2 (3.1 kbp), L3 (3.0 kbp), L4 (2.8 kbp), L5 (2.6 kbp) and M2 (1.3 kbp), which are changelessly present in diseased mushrooms. Additionally, the three minor dsRNA molecules, M1 (1.7 kbp), S1 (0.9 kbp) and S2 (0.8 kbp) can be also occasionally observed in the diseased mushrooms [25, 98, 66]. Since the previously identified 35 nm isometric virus particle was usually co-purified with the six major dsRNA elements and sometimes along with the minor dsRNAs from diseased mushrooms [44], this virus was firstly denominated as La France isometric virus (LIV) [67]. Later on, it was proposed to be renamed as *Agaricus bisporus* virus 1 (ABV1) due to its controversial role as the causative agent of the disease [42]. The presence of any cross

hybridization event between the nine La France dsRNAs was not observed even under stringent conditions [25]. This result indicated the uniqueness of each dsRNA element and refuted the hypotheses that they may be representatives of subgenomic or defective viral dsRNAs. The most plausible explanation is that the six major dsRNA molecules constitute the ABV1 genome, while the three minor dsRNAs, represented with less quantities, would be satellite dsRNAs [68]. Co-segregation of these dsRNA molecules during spore formation of the diseased mushrooms strongly suggests that their replications are interdependent and they compose a genome belonging to a single virus [67]. The complete nucleotide sequences of L1, L3, L5, M1 and M2 dsRNA molecules were determined [42, 26] and only the L1 dsRNA was shown to encode a putative RNA-dependent RNA polymerase [68]. Based on the phylogenetic analysis of the RdRp, encoded by AbV1 L1 dsRNA, the virus was found to be mostly related to the dsRNA virus family *Chrysoviridae* and officially declared to be in this family in the 9th ICTV Report. In *Agaricus bisporus*, AbV1 is often observed in a co-infection status with mushroom bacilliform virus (MBV) [63] [68] which was shown to have single strand RNA genome encoding an RdRp. Phylogenetic analysis performed with the MBV genome, linked this virus to the family *Barnaviridae*. No apparent phenotypic changes were observed in mushrooms solely infected with MBV [68]. The observation that the titre of MBV can be about 12 times higher in mushrooms coinfecting with AbV1 than it is in a single infection, [67] indicates a synergistic relationship between these viruses. This synergistic relationship may have some possible effects on the virulence of AbV1. Strict implementation of the viral hygiene guidelines minimizing the on-farm production and spread of infected basidiospores eventually brought the La France disease under control. Currently La France disease does not pose a threat to the mushroom industry.

During 1990s, another virus associated disease with multiple symptoms effecting *Agaricus bisporus* emerged in the mushroom farms of a number of European countries and was encountered with increasing incidences in the 2000s [60–62]. The symptoms of the disease includes sporophore growth inhibition or delay, brown discoloration of caps and malformed fruiting bodies. Before gaining any insight into the causative dsRNA elements or viral agents, a disease name Mushroom Virus X (MVX) was coined in order to collectively refer to these symptoms [15]. Nonetheless, the geographically segregated disease symptoms, i.e. sporophore growth retardation being more widespread in England whereas browning symptom was most often observed in other European countries including Netherlands, Belgium, Ireland and Poland, most probably indicate that different viral agents take part in the

development of different symptoms [5]. In fact, as the knowledge on browning symptoms of *Agaricus bisporus* accumulated, it was considered to be a distinct disease type and preferred to be designated as Brown Cap Mushroom Disease (BCMD) [12]. As similar with the La France disease, MVX disease was also shown to be transmitted vertically via infected basidiospores and horizontally through mycelial fragments [15, 22]. Detailed electron microscopy studies conducted with MVX specimens did not disclose any virus particle specifically associated with the disease (unpublished data) and this negative finding prompted the investigators to work on identifying non-encapsidated dsRNA elements responsible for the disease development.

In one study, primarily conducted for identifying dsRNA molecules that are specifically associated with MVX, dsRNA profiles of MVX diseased and healthy *Agaricus bisporus* samples collected from several mushroom farms in UK were compared [22]. As a result, 23 out of 26 newly identified dsRNA elements whose sizes range between 640 bp and 20.2 kbp were found to be associated with the disease conditions. Among these 23 MVX disease-associated dsRNA molecules, only four low molecular weight dsRNAs (^{MVX}0.6, ^{MVX}0.8, ^{MVX}1.8 and ^{MVX}2.0) were regularly linked with the occurrence of the browning symptom. The other tentative causal 19 dsRNAs were not able to be correlated with any specific MVX disease symptom. The highest number of dsRNAs observed in any one specimen was 17 in the same study [22]. In another study, *Agaricus bisporus* samples displaying MVX related browning symptoms were collected from various Dutch mushroom farms and analyzed for their dsRNA contents [79]. Similar to the previous study done by Grogan et al., five low molecular weight dsRNA molecules were determined and doubtfully associated with the brown discoloration status of the mushrooms in this study. Initially, only two of these 26 dsRNA elements, observed in MVX infected *Agaricus bisporus* specimens, were sequenced; the fully sequenced 14.5 kbp dsRNA identified as an Endornavirus [46] and the partially sequenced 17 kbp dsRNA which somewhat exhibited sequence similarity to *Cryphonectaria parasitica* hypovirus [79]. In a more recent study carried out by Eastwood et al., differential expression of host as well as non-host transcript fragments between brown cap mushroom diseased and non-diseased *Agaricus bisporus* samples were investigated using the suppression subtractive hybridization technique [12]. As a result, Ten low molecular weight non-*Agaricus* transcripts were found to be expressed with significantly higher fold changes (148–4019 fold) in infected mushrooms compared to non-infected. In the light of these hybridization assay results, the actual infective unit of BCMD was proposed to be 2.0 and 1.8 kbp dsRNA molecules which would represent the typical bisegmented genomes of a Partitivirus member

[12]. If that is the case, whether a partitivirus encoded coat protein plays a role in the development of this disease is uncertain since virus particles have not hitherto been observed in *Agaricus bisporus* with BCMD. In this study, viral transcripts were demonstrated to be expressed at two different levels in brown infected and white infected *Agaricus bisporus* fruit bodies attached to the same mycelial network. Compared to non-infected mushrooms, while moderate viral transcript increment (10^2 – 10^3 fold change) were observed in non-symptomatic infected mushrooms, highly symptomatic (brown colored) mushrooms included much more abundant viral transcripts (10^4 – 10^6 fold increase). According to the Eastwood et al. [12] this finding may indicate a spatially separated transition in viral life strategy from persistent to acute infection the causative factors of which are currently unknown. It was proposed in the same study that this step change in the viral activity would be affected by the phase III composting process used commonly in the mushroom industry. Phase III composting involves mycelial growth followed by cell breakage and cell mixing which increase the cell division and hyphal anastomosis. This composting stage may presumably facilitate the spread of viral elements via mycelial network. Lastly in the same study, some *Agaricus* host transcripts were shown to be significantly up-regulated in infected mushrooms including centrosome microtubule binding domain which might have roles in binding viral RNAs and viral proteins, DNA directed RNA polymerase that most probably indicates a change in host transcriptional pattern and one enzyme delta-1-pyrroline-5-carboxylate dehydrogenase which may take place in the redistribution of nutritional resources in the cap tissue [12]. Interestingly, none of the 26 genes responsible for melanine biosynthesis which could regulate brown discoloration were significantly up-regulated in mushrooms with BCMD. Thus, host factors that determine viral RNA levels and disease symptom expression have yet to be revealed.

Quite recently, the viral diversity of *Agaricus bisporus* was revealed using next generation sequencing of RNA extracts obtained from both diseased and non-diseased mushroom fruit bodies [9]. As a result of this deep sequencing and subsequent bioinformatic analyses, 15 unique RNA viruses (Tables 1, 2) and eight ORFans (non-host RNAs with no apparent similarity to any previously known sequences) were identified [9]. Among these 15 unique viral sequences, 12 were found to be related to linear single stranded RNA viruses from various families and the remaining three were shown to have sequence similarities with hypoviruses (Table 1, 2) [9]. Two of the newly identified 15 distinct *A. bisporus* viruses (AbV6 and AbV16) appeared to have segmented genomes. AbV16 were proposed to be composed of four genome segments (AbV16 RNA1-4) based on the presence of 25 nucleotide

long common 3' motif on each segment. While AbV16 RNA1 encodes a type II RdRp, AbV16 RNA2 seems to encode a type I Vmethyltransferase domain. On the other hand, AbV16 RNA3 and AbV16 RNA4 were thought to encode ORFs with unknown function. The phylogenetic analysis of AbV16 genome segments did not associate this virus to any currently present virus families. Thus a new virus family denominated as *Ambsetviridae* were proposed for the classification of this virus. AbV16 genome segments were found at high abundance in fruit bodies of *A. bisporus* with browning symptoms and considered to be one of the causative viral agents of the BCMD [9].

Viruses of *Pleurotus* spp

In *Pleurotus ostreatus* the presence of spherical and bacilliform viruses were demonstrated in malformed fruit bodies; yet has not been characterized in detail [20, 56]. In symptomless *Pleurotus cornucopiae* (Paulet) Rolland (Syn.: *Pleurotus sapidus* Sacc.) sporophores, spherical and rod shaped virus particles having dsRNA genome were described by Pingyan et al. [59]. Van der Lende et al. Defined isometric virus particles with different sizes and dsRNA elements in slowly growing cultivated *Pleurotus ostreatus* [43]. In this study, diseased oyster mushrooms were shown to host 7 different dsRNA molecules. Among these dsRNAs, the 2.4 kbp dsRNA was observed simultaneously with infective viral particles varying in size and could be transmitted to virus-free protoplasts via transfection of these virus particles [43]. Nevertheless, the slow growth rate of diseased *Pleurotus ostreatus* could not be precisely correlated with the presence of dsRNA elements in this study. As opposed to most fungal viruses that have dsRNA genome, in *P. ostreatus*, Yu et al. [104] discovered a new 27 nm isometric virus having 5.784 kb long linear ssRNA genome and a capsid protein of roughly 28.5 kDa. This virus, named Oyster Mushroom Spherical Virus (OMSV), was strongly associated with severely epidemic oyster mushroom dieback disease. Another spherical virus containing linear, single stranded RNA genome with a size of approximately 7.8 kb was identified in cultivated mushroom *Pleurotus eryngii* (DC.) Quél. showing severe epidemic symptoms [64]. The virus was named *Pleurotus eryngii* Spherical Virus (PeSV) and its genome has yet to be sequenced. Lim et al. identified a new virus infecting *P. ostreatus* that has bipartite dsRNA genome exhibiting similarities to partitiviruses [45]. Unlike OMSV, this virus, named as *P. ostreatus virus 1* (PoV1), was not found to be associated with disease symptoms. Two additional 34 nm isometric virus particles containing dsRNA genome, named as Oyster Mushroom Isometric Virus 1 and 2 (OMIV-1 and OMIV-2), were identified in *Pleurotus ostreatus* and correlated with the dieback disease [104].

The phylogenetic analysis conducted with the RdRp coding segment of OMIV-2 revealed its similarity to partitiviruses (<http://talk.ictvonline.org>). Later on, the same research group described a new 43 nm isometric virus encapsulating four different dsRNA genome segments in diseased fruiting bodies of *Pleurotus ostreatus* and named it as Oyster Mushroom Isometric Virus (OMIV) [65]. Although the sequence information of this virus is lacking, it is expected to be a distinct virus species. In a more recent study, another novel, asymptomatic 33 nm isometric virus particle having a bisegmented dsRNA genome was isolated from the healthy sporophores of *Pleurotus ostreatus* Shin Nong strain [39]. The virus was named *Pleurotus ostreatus virus Shin-Nong* (PoV-SN) and the phylogenetic analysis carried out with the partial sequence of the RdRp encoding RNA-1 genome segment revealed its closest relatives as the *Helicobasidium mompa* Virus and the *Rosalina necatrix* Virus which are member of the family *Partitiviridae*.

Viruses of *Lentinula edodes*

The very first reports on the presence of viruses in Shiitake mushroom *Lentinula edodes* came in the early 1970s. Inouye et al. demonstrated three distinct types of viruses with isometric (35–36 nm), filamentous (15 nm × 200 nm) and rod-shaped (28 nm × 280–300 nm) particles in infected *L. edodes* with electron microscopy [35]. However these viruses were not further characterized. Later on, Ushiyama et al. [88] detected three morphologically different polyhedral virus particles with diameters of roughly 25, 30 and 39 nm in both sporophores and mycelia of *L. edodes*. As being the most prevalent one, the 39 nm virus particles were studied more in detail and revealed to be composed of an outer capsid shell and an inner core containing a dsRNA genome [88, 87]. Besides, this virus were mostly found to localize in the cytoplasm and in the vacuoles of hyphal cells, either with randomly dispersed or with aggregated forms [86]. In a study on determining the prevalence and diversity of dsRNA elements in *L. edodes* strains present in the US, 23 out of 25 genetically-diverse lines of *L. edodes* were shown to harbor dsRNA molecules with total of 14 different molecular weights [71]. However, the presence or absence of particular dsRNA element could not be correlated with any certain phenotypic characteristic and there were also no evidence of harmful effects associated with the presence of dsRNA elements in the studied *L. edodes* strains [71]. The only ssRNA virus known to infect *L. edodes* was reported by Xueren et al. [103]. These 34 nm isometric virus particles having a capsid composed of single 22 kDa capsid protein were isolated from the diseased mycelia and shown to contain a ssRNA genome with a length of approximately 7.3 kb [103]. Due to the absence of sequencing technologies at the time, these early studies of *L. edodes* viruses did not include any

sequence data and thus far the genomes of these discovered viruses and the isolated dsRNA elements have not been sequenced.

Among the viruses of *L. edodes*, *L. edodes* mycovirus HKB (LeV-HKB) was the first whose genome has been fully sequenced [49]. LeV-HKB was isolated from an asymptomatic *L. edodes* strain and revealed to be a linear unencapsidated dsRNA element by direct imaging of the virus using atomic force microscopy. The genome of LeV-HKB is 11,282 bp long and contains two open reading frames (ORF1 and 2). While ORF1 coded for a hypothetical protein bearing a NUDIX domain (a member of pyrophosphohydrolases), the ORF2 was shown to encode a putative RdRp [49]. In another recent study, a 55 nm spherical virus particle containing a roughly 12 kb long dsRNA genome was identified in a diseased strain of *L. edodes* and named as *L. edodes* spherical virus (LeSV) [100]. The subsequent phylogenetic analysis based on part of its genome and N-terminal sequence of its capsid protein revealed that LeSV is closely related to LeV-HKB and to the previously identified *L. edodes* mycovirus HKA (LeV-HKA) [53]. The closest relatives of these viruses appeared to be *Phlebiopsis gigantea* mycovirus dsRNA 1 (PgV) and based on phylogenetic analysis they were initially suggested to be in the recently defined family *Megabirnaviridae* by Wang et al. [99] though the members of this family are characterized by containing bipartite dsRNA genome. More recently, a newly proposed genus *Plegivirus* (acronym from the host of PgV *P. gigantea*) was offered for the classification of LeV-HKA, LeV-HKB and LeSV along with the newly identified dsRNA virus, in mycorrhizal macrofungus *Thelephora terrestris* Ehrh. [58]. Quite recently, a partitivirus was identified in a diseased *L. edodes* strain and named as *Lentinula edodes* Partitivirus 1 (LePV1) [23]. The virus was shown to have a isometric virus particles with a diameter of 34 nm and bisegmented dsRNA genome with the sizes of 2382 and 2245 bp designated as LePV1 dsRNA-1 and LePV1 dsRNA-2 respectively. Phylogenetic analysis based on the amino acid sequence of LePV1 dsRNA-1 coded RdRp showed that LePV1 is mostly related to the Grapevine Partitivirus found in the genus *Betapartitivirus*. Besides, when compared to the strain infected with LePV1 alone, significantly more LePV1 dsRNA-1 was observed in the strain co-infected with LeV-HKB which pointed out that the expression of LeVP1 RdRp is promoted by LeV-HKB [23]. Although this study did not present direct evidences that LeVP1 is the causative agent of the observed disease symptoms including degeneration of mycelia and imperfect browning, the authors noted that the infected symptomatic strain was found to harbor much more abundant LeVP1 dsRNAs than

infected asymptomatic strain used in this study [23]. This phenomenon with unknown mechanism may also result from a step change in the viral activity from slow, persistent replication strategy to acute as proposed for viruses causing BCMD in *A. bisporus* [12].

Viruses of *Flammulina velutipes*

Flammulina velutipes also known as Enokitake or Enoki is one of the most widely produced commercial mushroom species in east Asia. The two commonly observed deteriorative symptoms of this mushroom were reported to be (1) spontaneous brown discoloration in fruit bodies and (2) reduced production or malformation of fruit bodies [47]. In the first research study carried out for determining the presence of viruses or viral elements in *F. velutipes*, two dsRNA elements associated with a 50 nm virus particles were identified in the mycelium from a variant of *F. velutipes* commercial strain with spontaneously brown-colored fruit body [47]. Since this virus was absent from the other variants of the studied *F. velutipes* strain exhibiting no symptoms or different debilitating symptoms, it was proposed to be the casual agent of *F. velutipes* browning symptoms [47].

Long after this study, the virus was further characterized by the same research group and named as *Flammulina velutipes* Browning Virus (FvBV) [48]. The 1925 bp long dsRNA1 and 1730 bp long dsRNA2 were completely sequenced and was found to encode a putative 66 kDa RdRp and a 60 kDa coat protein (CP) respectively. Phylogenetic analysis of the dsRNA-1 coded RdRp sequence showed FvBV to be a Partitivirus and its closest relative was found to be *Chondrostereum purpureum* cryptic virus 1 [48]. In this study, 14 different wild *F. velutipes* strains were collected from various regions of Japan and subjected to RT-PCR analysis based on the detection of CP coding FvBV RNA2. As a result, Three isolates obtained from central parts of Japan displaying browning symptoms were found to harbor FvBV [48]. Although Koch's postulates were not applied in this study, perhaps, this virus species represents a rare case among Partitiviruses infecting fungi owing to its apparent association with a well defined phenotypic change in *F. velutipes*.

Furthermore, in that same study, a distinct isometric virus named as *Flammulina velutipes* Isometric Virus (FvIV) was reported to be identified in one of the studied wild strains of *F. velutipes* suffering from growth retardation with flat morphology. The phylogenetic analysis of the FvIV encoded putative RdRp revealed this virus to be a member of Partitivirus with its most close relative as *Sclerotinia sclerotiorum* Partitivirus S [48].

Viruses of *Tuber* spp

Despite their ecological importance, research on viruses of ectomycorrhizal macrofungi has only been sparsely conducted. *Tuber aestivum* and *Tuber excavatum* are the first fungal species among the ectomycorrhizal macrofungi the viruses of which were identified and characterized; though an uncharacterized dsRNA element was previously demonstrated in *Hebelome circinans* [1]. The first virus identified in the truffle Genus *Tuber* was a Totivirus isolated from black noble truffle *T. aestivum* [85]. This Totivirus was named Tuber aestivum Virus 1 (TaV1) and shown to have a single and linear, 4587 bp long dsRNA genome with two ORFs (ORF1 and ORF2) encoding for a capsid protein (CP) and an RdRp respectively. Phylogenetic analysis conducted based on the sequences of both TaV1 CP and RdRp revealed this virus to be most closely related to the Totivirus *Saccharomyces cerevisiae* Virus L-A (ScVLA) with the sequence identities of 39.1% between CPs and 48.7% between RdRps [85].

The second virus identified in *T. aestivum* was an *Endornavirus* named as Tuber aestivum Endorna Virus (TaEV) [83]. The unencapsidated, linear, 9760 bp long dsRNA genome was shown to have a single ORF encoding for a putative viral methyltransferase (VMet), a DEAD-like helicase (DEXDc) and an RdRp. This type of *Endornavirus* genome organization was inferred to be specific to Endornaviruses infecting Ascomycota as Endornaviruses of Basidiomycota appear to have two common sequence motifs encoding for viral helicase (VHel) and UDP-glycosyltransferase (UGT). Phylogenetic analysis of the TaEV encoded VMet, DEXDc and RdRp indicated that TaEV is most closely associated with *Gremmeniella abietina* type B RNA virus XL1 and 2 [83].

Lastly, a *Mitovirus* infecting *T. aestivum* was identified and characterized by Stielow et al. [84]. The virus named as Tuber aestivum Mitovirus (TaMV) was revealed to have the largest genome among the Mitoviruses identified so far. The 3480 nt long, linear, ssRNA genome was shown to have a single ORF encoding a 635 amino acid long RdRp with the predicted molecular weight of 71.3 kDa. The closest relative of TaMV was revealed to be *Ophiostoma Mitovirus 1b* according to the phylogenetic analysis of the ORF encoded RdRp and the overall nucleotide sequence. TaMV was also shown to possess the largest 3' UTR (952 nt) among Mitoviruses. Based on the observation that the mitoviruses with extremely long 5' and 3' UTRs tend to cluster together, led to the hypothesis that mitoviruses might have evolved from two distinct viral ancestor; nonetheless there are exceptional mitoviruses that do not fit to this rule [84]. In *T. excavatum*, another mitovirus with a genome size of 3305 nt was found and characterized [82]. Different than TaMV, this virus was revealed to have a

large 5' UTR and a quite short 3' UTR. The virus was named Tuber excavatum Mitovirus (TeMV) and its most closely related relative was found to be *Glomus* sp. RF1 Small Virus based on the phylogenetic analysis of TeMV coded RdRp [82].

Viruses of *B. edulis*, *L. tabidus*, *L. rufus*, *A. aegerita* and *V. volvacea*

In a research study, basically aimed to reveal the causative agent(s) of a well defined “little cap disease” observed in *Boletus edulis*, which resembles the La France disease of *A. bisporus* and characterized by sporophores with small caps and thick stipes, both diseased and healthy isolates of *B. edulis* were examined with electron microscopy for the presence of virus particles [30]. As a result, three polyhedral virus particles roughly 28, 33 and 42 nm in diameter and one filamentous virus particle with a length of about 500 nm and a width of about 13 nm were identified. However, since these entire virus particles were observed in both diseased and healthy sporophores of *B. edulis*, none of them were able to be linked to the disease condition conclusively and neither of these were later characterized in detail [30]. Whether virus like symptoms of little cap disease in *B. edulis* is caused by any of these viruses or results from infection with unencapsidated RNA viruses remains unknown.

In a recent study primarily conducted for determining the transmission rates of *Heterobasidion* viruses to the distantly related fungal species inhabiting a same forest site, two dsRNA viruses were identified in the ectomycorrhizal macrofungal species *Lactarius tabidus* and *Lactarius rufus* using an high throughput sequencing approach [95]. These viruses were designated as LtRV1 and LrRV1 referring to *Lactarius tabidus* RNA Virus 1 and *Lactarius rufus* RNA Virus 1 respectively. The strains of LtRV1 and LrRV1 exhibited approximately 79–82% identity with each other at the nucleotide level and phylogenetic analysis based on the amino acid sequence of LtRV1 and LrRV1 encoded RdRps indicated an approximately 46–47% similarity of these viruses with *Sclerotium hydrophilum* Virus 1 a member of the family *Partitiviridae* [95].

Investigations of viruses and viral elements in commercial mushroom *Agrocybe aegerita* using density gradient ultracentrifugation revealed a cytoplasmic, icosahedral virion with an approximate diameter of 26 nm that possesses a single, linear dsRNA genome with a size of about 6.2 kbp [2]. The virus was considered to be asymptomatic as it was isolated from a healthy strain of *A. aegerita*. This virus was speculated to be a member of the family *Totiviridae* based on the virion structure and genome size. However, its dsRNA genome has not been sequenced and hence, its precise classification has not been

made yet. In the same study, the investigators also identified three, naked dsRNA elements, differing in size from ~ 1.7 to ~ 1.9 kbp, in the membranous vesicle fractions however they were not characterized further [2].

In the other cultivated mushroom *Volvariella volvacea*, commonly known as straw mushroom, a polyhedral, 35 nm virus particle containing an approximately 9.3 kb long, linear dsRNA genome was identified [6, 7]. The virus was named *Volvariella volvacea* Virus (VvV). This virus was isolated from a healthy isolate of *V. volvacea* and thus regarded as asymptomatic. The dsRNA genome of VvV have not been sequenced thus far and therefore the taxonomic category this virus belongs to have not been determined yet. However, the relatively long and non-segmented genome associated with a particle implies a dsRNA virus with novel family.

Other macrofungal viruses

Relatively recently, a 3765 bp long, apparently unencapsidated dsRNA virus was identified in an isolate of non-cultivated *Agaricomycotina*, *Clitocybe odora* (Bull.) P. Kumm. exhibiting distorted morphology [27]. The absence of this dsRNA virus from the healthy *C. odora* isolates indicated a correlation between this virus and abnormal fruiting body with reduced cap or morel-like shape. This virus was named *Clitocybe odora* Virus 1 (CoV1) and bioinformatic analysis revealed it to contain two open reading frames (ORF 1 and 2). While the ORF 1 was found to encode for a putative 102.3 kDa RdRp, the ORF 2 was proposed to encode for a 20.2 kDa capsid protein deduced from its apparent calcium binding domain. However, if the mitochondrial codon usage of filamentous fungi is taken into account, the opal stop codon (UGA) resides at the end of the ORF 1 was inferred as a potential readthrough codon and thought to connect the two ORFs leading to a production of 128.25 kDa polyprotein [27]. Phylogenetic analysis of the ORF 1 encoded RdRp clustered CoV1 with *Thanatephorus cucumeris* Virus a member of mitovirus. In addition to having sequence homology, CoV1 also exhibited a similar GC content and codon usage with *Thanatephorus cucumeris* Virus. However, while a DNA form was proposed for *Thanatephorus cucumeris* Virus, such a DNA stage was not identified for CoV1. Furthermore, although no virus particle was associated with CoV1, the ORF 2 which is present in the CoV1 genome appears to code for a capsid protein and this ORF is absent from *Thanatephorus cucumeris* Virus. Thus, CoV1 seems to represent a member of virus taxa that has yet to be defined.

Quite recently, an asymptomatic virus which seems to be unencapsidated and consists of 10,316 bp long naked dsRNA was identified in the mycorrhizal macrofungi

Thelephora terrestris [58]. The virus was named *Thelephora terrestris* Virus 1 (TtV1) and shown to contain two separate open reading frames (ORF1 and 2) encoding for a 202 and a 174 kDa hypothetical proteins respectively. Also, a possible translation of ORF2 as a fusion protein with ORF1 was inferred from the presence of ribosomal-1 frameshifting sequence (AAAAAAA) upstream of the ORF2 start codon. Furthermore, bioinformatic analysis of the TtV1 revealed a 2A protease like motif and a NUDIX pyrophosphohydrolase motif on the upstream of ORF1 and a Phytoreovirus S7 domain on the upstream of ORF2. Phylogenetic analysis made based on the sequence of ORF2 encoded RdRp grouped TtV1 into a distinct clade along with *Phlebiopsis gigantea* Large Virus 1 (PgLV1) and *Lentinula edodes* mycoviruses LeVHKB and LeVHKA. Therefore, due to the obviously separate phylogenetic status of the TtV1, PgLV1 and LeV, a new genus named *Phlegivirus* containing these viruses was proposed. Lastly, the presence of TtV1 was demonstrated in the two (*Steganoecarus carinatus* and *Nothrus silvestris*) of the 20 examined oribatid mite species inhabiting the vicinity of *T. terrestris* though the roles of mites in fungal virus ecology is in many aspects ambiguous [58].

In an ascomycota species *Peziza ostracoderma* Korf, rod shaped virus particles were demonstrated in both cell free extracts and ultrathin sections of healthy sporophores using electron microscopy [10]. The virus was also shown to be structurally and functionally distinct from Tobacco Mosaic Virus, yet was not further characterized. By looking at its structural features, it appears that this virus might be a member of either the Benyviridae or Virgaviridae family.

Lastly, an isometric virus particle with an approximate diameter of 130 nm was identified in the “hare’s foot inkcap mushroom” *Coprinopsis lagopus* (Fr.) Redhead, Vilgalys & Moncalvo by the aid of electron microscopy. However a limited data was generated regarding this virus when considering the time the study was conducted and therefore its phylogenetic characteristics are not known [76].

Concluding remarks

Considering the approximate total number of macrofungal species which is the sum of roughly 22,000 known species and 53,000–110,000 unknown species, it can be estimated that the number of undiscovered macrofungal viruses present in nature is even much greater. These high macrofungal virus diversity is to be studied and revealed using next generation sequencing and relevant bioinformatic tools. In this sense, as long as the new sequencing technologies become widely available, we can foresee a considerable increase in the sequence data of macrofungal viruses in the near future.

Currently, very few information is available in regards to the replication and gene expression strategies of macrofungal viruses. These molecular mechanisms should be studied using transfection methods and particularly using reverse genetic approaches. Besides, in order to get more insight into the virus induced phenotypic changes observed in infected macrofungi, molecular and genetic basis of macrofungal virus host interactions needs to be elucidated both at the transcript and protein levels. Studying such interactions are essential for comprehensively understanding the virus induced pathogenesis of diseases seen in the economically important macrofungi and developing feasible prevention strategies. Revealing the mechanisms of host–pathogen interactions in mycovirus-fungus systems is also crucial for developing more potent biocontrol agents as in the case of *Heterobasidion* viruses.

Other than *Heterobasidion* viruses, much less is known regarding the persistence, transmission, and effects of macrofungal viruses in fungal populations present in nature. Due to the legitimate safety reasons, studying the ecology of fungal viruses becomes more of an issue especially for those used as biocontrol agents in nature. On the other hand, improvement of the potency of mycovirus based biocontrol agents would also rely on developing recombinant virus strains. As long as the virulence factors of a given phytopathogenic fungus is known, it would be possible to target them with a transgene expressed by its related biocontrol agent. In the case of *Heterobasidion* viruses which mostly include members of *Partitiviridae*, the small genome and virion size may pose an obstacle to the use of relatively large transgenes. Perhaps, to this end, constructing recombinant *Heterobasidion* viruses that operate RdRp directed expression of siRNAs targeting virulence factors, may be more plausible and appropriate for these applications.

In the decade of destructive Ebola and Zika epidemics, we see helpful to remind that not all viruses are harmful and in this respect, macrofungal viruses deserve a special interest not only from the economical stand point but also in the context of unveiling their diversity and ecology.

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