



# Shedding light on mushroom viruses

A complex of novel viruses was discovered in commercial mushrooms in the early 2000s, which reduced the quality of this premium horticultural product across Europe. **TEAGASC**- and EU-funded research has now characterised the viruses and shown that the *Agaricus bisporus* mushroom is a model system to study fungal viromes.

## Mushroom virus disease

Mushrooms are a high-value export crop, with an annual production value in Ireland in the region of €130 million. They can suffer from economically damaging viral diseases such as La France disease, which emerged in the 1960s, and which has largely disappeared from modern farms due to increased hygiene levels. It was a big surprise therefore when virus-like symptoms started to recur in the late 1990s and 2000s, but mushrooms were negative for the 35 nanometre virus particles associated with La France disease. The term Mushroom Virus X (MVX) disease was coined to cover a range of symptoms, which included widespread brown discolouration of what should be pristine white mushrooms, rendering them unmarketable (**Figure 1**). MVX has been associated with at least 26 double-stranded RNAs (dsRNAs), which are assumed to be unencapsidated viral genomes as no viral particles have been observed.

## Virus-infected mushrooms analysed

The lack of knowledge on the identity and characterisation of the MVX dsRNAs has impeded our ability to develop good diagnostics for the industry, as well as to understand their role and function within the mushroom mycelium. RNA was isolated from ten samples of mushrooms from different sources and with different viral symptoms. Viral contigs were *de novo* assembled, sequenced and classified to shed light on their relatedness and phylogeny, with reference to known virus families. Differences in viral number, titre and composition were identified, suggesting different host adaptations and viral lifestyle transitions.

## Eighteen mushroom viruses identified

Next-generation sequencing of the RNA extracted from the mushroom samples revealed 30 distinct RNA contigs not found in the *Agaricus bisporus* genome, which ranged in size from 0.5-14.5kb. Eighteen of

them contained an RNA-dependent RNA polymerase (RdRp) domain, characteristic of fungal viruses, and we propose that these represent 18 distinct viruses (**Figure 2**). A nomenclature is proposed of *Agaricus bisporus* Virus N (N replaced by a sequential number). Sixteen of the viruses had a monopartite structure of a single RNA molecule while two viruses were segmented viruses: AbV6 consisted of two separate contigs, while AbV16 contained four separate contigs. AbV16 is the fungal virus associated with the brown mushroom symptoms seen in crops affected by MVX. The RdRps of the 18 viruses have closest amino acid homology to a diverse array of positive sense single-stranded RNA viral orders/families/genera (ss(+)RNA): *Hypoviridae*, *Tymovirales* (*Betaflexiviridae* and *Gammalflexiviridae*), *Narnaviridae*, *Barnaviridae*, *Bromoviridae*, *Endornaviridae*, *Virgaviridae*, and the unassigned genera *Benyvirus* and *Ourmiavirus*. The majority of the viruses are only distantly related to known viruses and, accordingly, new phylogenies are proposed. Phylogenetic analysis of the RdRp domain of the AbV16 virus places it into its own unique clade, distinctive from all previously described viral groupings. Therefore, we propose a new viral family, which has been named *Ambsetviridae*. Bioinformatic analysis of existing RNA sequence databases reveals the existence of other members of the *Ambsetviridae* family from the plant and fungal kingdoms.

## Viral interactions

Only AbV16 has been directly correlated with the adverse browning symptoms displayed by mushrooms and it would appear that the other viruses may be benign. There is evidence that AbV16 can be 'lost' during crop cultivation while other viruses persist, as individual mushrooms attached to the same mycelial network can manifest different levels of AbV16. It is interesting to speculate that within the mycelial network there is a dynamic interaction (competition?) between the different viruses. We have observed changing patterns of viral composition from



FIGURE 1: Poor-quality discoloured mushrooms.

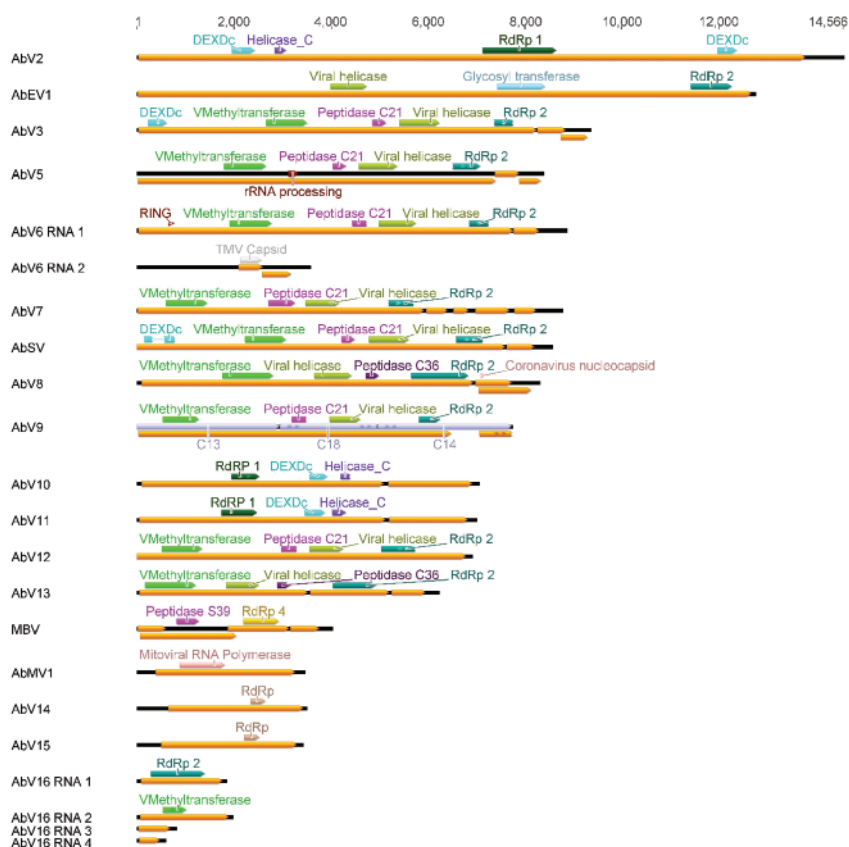


FIGURE 2: Genome organisation and protein coding potential of the 18 viruses identified in the MVX complex (from Deakin et al., 2017).

sample to sample, with a consequential changing pattern of cap browning symptoms when AbV16 titre increases or decreases. This hypothesis goes some way towards explaining the variable pattern of symptom expression that has been observed in commercial crops for the past 20 years. It may be that one or more of the other viruses can outcompete or suppress AbV16 within the mycelial network, something which could be useful from a virus resistance perspective. Current work is exploring this hypothesis.

The presence of so many viruses in a single organism is relatively rare and thus *Agaricus bisporus* is an ideal model organism to study viral-viral and viral-host interactions of a fungal virome. Full details of this work can be found in the scientific publication of the results (Deakin, Dobbs *et al.*, 2017).

### Significance for the mushroom sector

The genetic sequence and identity of 18 new fungal viruses that can infect commercial mushroom crops is now known. The presence of these viruses can be detected by molecular methods in a variety of mushroom material, such as mushrooms and compost, while mushroom breeders can screen fungal germ plasm for viruses during strain development.

### Reference

Deakin, G., Dobbs, E., Bennett, J., Jones, I.M., Grogan, H.M. and Burton, K.S. (2017). 'Multiple viral infections in *Agaricus bisporus* – Characterisation of 18 unique RNA viruses and 8 ORFans identified by deep sequencing.' *Scientific Reports*, 7, Article number: 2469. Available from: <http://www.nature.com/articles/s41598-017-01592-9>.

### Acknowledgements

Julie Bennett of NIAB EMR, UK, and Ian Jones, University of Reading, UK, also contributed to this work. The research leading to these results has received funding from Teagasc and from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 286836 (MushTV).

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