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Characterisation of new viruses affecting commercial mushroom crops



Key external stakeholders:

Mushroom industry, Mushroom breeders, Virologists

Practical implications for stakeholders:

A new mushroom virus disease (Mushroom Virus X) has affected commercial mushroom production in Europe since the early 2000's. It causes white mushrooms to develop various degrees of brown discoloration during growth or post-harvest, resulting in quality downgrading and product rejection. In this study 30 different viral RNAs from *A. bisporus* mushroom fruitbodies were sequenced, characterised and classified phylogenetically.

- Bioinformatics analysis identified up to 24 viral RNAs in samples with disease symptoms and 8-17 in asymptomatic samples
- The fungal virus linked to the brown cap discoloration symptoms in mushrooms has been identified as **AbV16** - a multi partite virus with four elements.
- Most of the viruses in the MVX complex appear to be benign and of unknown activity but they would still be considered as undesirable in mushroom spawn.

Main results:

- Eighteen viral contigs contained an RNA dependent RNA polymerase (RdRp) domain and we propose that these represent 18 distinct viruses. A nomenclature is proposed of **Agaricus bisporus Virus N** (N replaced by a sequential number). The majority are only distantly related to known viruses, and accordingly new phylogenies are proposed.
- The amount of **AbV16** virus correlated with brown cap discoloration symptoms. It is a multipartite virus with four separate elements. It forms a unique clade and has been placed in a new viral family which has been named the *Ambsetviridae*.
- To our knowledge this is the first *de novo* deep sequencing study of a large number virome for a single organism.

Opportunity / Benefit:

- The mushroom industry is more informed on the prevalence and identity of novel fungal viruses that can infect mushrooms. and for which molecular diagnostics are available.
- Mushroom breeders can screen wild *Agaricus* germ plasm for the presence of fungal viruses.
- *Agaricus bisporus* offers a model system to study fungal-viral and viral-viral interactions.

Collaborating Institutions:

NIAB EMR, Kent, UK. University of Reading, UK. Teagasc, Ashtown, Dublin 15

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1. Project background:

Agaricus bisporus, the cultivated white mushroom, is commercially grown via vegetative propagation. It is a high value, export based, horticultural crop, with an annual production value in Ireland in the region of €130 million. *Agaricus bisporus* can suffer from economically-damaging viral diseases such as La France disease, which emerged in the 1960s's, and Mushroom Virus X (MVX) disease, which emerged in the 2000's. La France disease is caused by *Agaricus bisporus* Virus 1 (AbV1), and is associated with 9 viral elements within a 35 nanometre particle. MVX disease is a collective name for a mixture of symptoms (fruitbody browning, crop delay, poor quality and distorted mushrooms) and is associated with 30 dsRNAs. These RNAs are assumed to be unencapsidated viral genomes as no viral particles have been observed. Profoundly different levels of the low molecular weight RNA species (> 10³ fold difference) have been observed in adjacent non-symptomatic and symptomatic mushrooms attached to the same mycelial network, indicating a spatially-separated, viral life-style that transitions between persistent and acute.

The extent to which the sequences of the 30 viral RNAs of MVX are related to each other, for example as defective or satellite RNAs, or to other viruses, was unknown. Two reports have presented evidence of hybridisation of two or more RNAs of the MVX complex with a single probe. Only two published RNA sequences have been associated with the MVX complex; the 14.5 kbp dsRNA identified as an Endornavirus, AbEV1, and the partial sequence of a 17 kbp dsRNA hypothesised as a hypovirus.

Sequencing and phylogenetic classification of the multiple viruses associated with *A. bisporus* has enabled a greater understanding of their relatedness, interactions and dynamics, host adaptations and viral life-style transitions. In this study 30 different viral RNAs from *A. bisporus* mushroom fruitbodies were sequenced, characterised and classified phylogenetically in order to progress our understanding of mushroom viruses and associated symptomologies.

2. Questions addressed by the project:

- What is the genome sequence and identity of thirty putative RNA viruses that have been detected in MVX infected mushrooms?
- Can the viruses be characterized and classified phylogenetically?
- What can be learned from studying this large and variable number of viruses in a single organism?

3. The experimental studies:

Next Generation Sequencing was performed on RNA extracted from ten samples of *Agaricus bisporus* mushroom fruitbodies (midrange hybrid white strains). Three of the samples were of diseased mushrooms displaying the fruitbody browning symptom depicted in the image on Page 1, and which were collected from different mushroom farms in 2004 and 2011. Six further samples were from mushroom crops grown at Teagasc's Mushroom Unit that had been inoculated with virus-infected mycelial sub-cultures taken from diseased fruitbodies (which had displayed a range of viral disease symptoms) collected between 2000–2004. Fruitbodies from the inoculated crops were non-symptomatic and did not develop the mushroom browning symptom. In addition fruitbodies were grown and collected from non-diseased commercial cultures at Teagasc's Mushroom Unit. All fruitbodies were frozen in liquid nitrogen and stored at -80°C until use. RNA extracts were prepared and tested for purity using standard methods.

De novo assembly and virus characterisation were carried out using standard software and bioinformatics. Full details of methods, including the virus discovery pipeline and phylogenetic analysis, can be found in the scientific publication of the results at <http://www.nature.com/articles/s41598-017-01592-9>.

4. Main results:

- Ten dsRNA-enriched samples extracted from mushroom fruitbodies were sequenced and 30 viral contigs were *de novo* assembled. Sanger sequencing revealed 30 distinct RNA molecules not found in

the *A. bisporus* genome which ranged in size from 0.5kb – 14.5kb.

- Eighteen contigs contained an RNA dependent RNA polymerase (RdRp) domain and we propose that these represent 18 distinct viruses (Figure 1). A nomenclature is proposed of **Agaricus bisporus Virus N** (N replaced by a sequential number).



Figure 1. Diagrammatic representation of the genome size (in bases), organisation and protein coding potential of the viruses (from Deakin, Dobbs *et al.* 2017).

- Sixteen of the contigs were consistent with monopartite viruses. In addition we propose two, novel, segmented viruses: (1) AbV6 consisting of two separate contigs and (2) **AbV16** consisting of four separate contigs.
- AbV16** is the fungal virus associated with the brown mushroom symptoms seen in crops affected by Mushroom Virus X.
- The RdRps of the 18 viruses have closest amino acid homology to a diverse array of positive sense single-stranded RNA (ss(+))RNA viral orders/families/genera: *Hypoviridae*, *Tymovirales* (*Betaflexiviridae* and *Gammaflexiviridae*), *Narnaviridae*, *Barnaviridae*, *Bromoviridae*, *Endornaviridae*, *Virgaviridae*, 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 data-bases reveals the existence of other members of the *Ambsetviridae* from the plant and fungal kingdoms.

5. Opportunity/Benefit:

The genetic sequence and identity of up to 30 new fungal viruses is now known. The presence of these viruses can be detected by molecular methods in a wide variety of mushroom material. The mushroom industry can monitor for virus presence in mushrooms and compost while mushroom breeders can screen fungal germ plasm for viruses during strain development.

Additionally, improved sequencing of host and virus genomes has suggested that multiple virus infections of eukaryotes may be more common than previously indicated. *Agaricus bisporus* has been shown to host up to 30 viruses, making it an ideal model organism with which to study potential viral-viral and fungal-viral interactions of a fungal virome.

▪ Dissemination:

This work contributed to Work Package 2 of EU FP7 project MushTV (286836), which had a consortium of 17 partners, and results were disseminated to a wide variety of audiences as a result. Workshops were organised by mushroom grower associations and compost producers in Ireland, United Kingdom, the Netherlands, Belgium and Poland in February and March 2015. A number of in-house seminars/workshops were given on request to staff of some beneficiaries in the final months of the project. A number of general dissemination articles were published during the course of the project for both the wider mushroom industry as well as for more general audiences. Scientists involved directly in the research gave scientific and other presentations at various scientific meetings, conferences and events.

Main publications:

- Deakin, G., Dobbs, E., Bennett, J., Jones, IM, Grogan, HM & Burton, KS. (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 doi:10.1038/s41598-017-01592-9 (<http://www.nature.com/articles/s41598-017-01592-9>)
- Fleming-Archibald, C., Ruggiero, A., Deakin, G., Dobbs, E., Burton, K. and Grogan, H. (2016). Characterisation of Viruses Associated with Mushroom Virus X (MVX) and their Detection within the European Mushroom Industry. (Proc. 19th. ISMS Int. Con. Amsterdam, 30 May – 2 June, Amsterdam, 2016). *Mushroom Science IX*. Science and Cultivation of Edible Fungi, pp. 109-113.
- Dobbs, E., Deakin, G., Bennett, J., Fleming-Archibald, C., Grogan, H., Jones, I. and Burton, K. (2016). A sensitive and quantitative test for >31 viral RNAs associated with the Mushroom Virus X diseases in both compost and mushrooms. . (Proc. 19th. ISMS Int. Con. Amsterdam, 30 May – 2 June, Amsterdam, 2016). *Mushroom Science IX*. Science and Cultivation of Edible Fungi, pp. 75-81.Xx
- Deakin, G. (2015). Understanding the biology of Mushroom Virus X by molecular characterisation, location and translocation of RNA and their role in disease epidemiology. Ph.D Thesis, University of Reading, UK.

Popular publications:

- Grogan, H., Kilpatrick, M., Deakin, G. and Burton, K. (2015). Advances in mushroom science – viruses and Trichoderma. *TResearch* Vol 10(3); 20-22. <https://www.teagasc.ie/media/website/publications/2015/TResearch-Autumn-15.pdf>
- Fleming-Archibald, C., Burton, K. and Grogan, H. (2015). MushTV Factsheet 02/15. Brown Cap Mushroom Virus (associated with Mushroom Virus X) prevention. <https://www.teagasc.ie/publications/2015/brown-cap-mushroom-virus-prevention.php>
- Grogan H (2015). MushTV – Providing IPM solutions to the industry. *Mushroom Business*, Vol 70, April 2015, pp. 20-22
- Grogan H, & Kilpatrick, M. (2015). Collaborative research means maximum benefit for mushroom Industry. *Politics First*, Volume 5, Issue 21, July 2015, Page 78

6. Compiled by: Helen Grogan

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