Mushroom viruses and *Agaricus* genomics

TEAGASC researchers have been examining how mushroom strains respond to virus infections, guiding efforts to develop new cultures that are either tolerant of or resistant to infection.

The commercial mushroom, *Agaricus bisporus*, is susceptible to a disease caused by a complex of viruses known collectively as mushroom virus X (MVX). Symptoms of MVX include poor-quality mushrooms and mushroom cap discolouration (browning), which are correlated with *A. bisporus* virus 16 (AbV16). Another virus, AbV6, is also associated with virus outbreaks but has not been directly correlated with distinct symptoms. Most modern-day commercial varieties of *A. bisporus* are mid-range hybrids, and are almost genetically identical, having been derived from a hybrid cross between two strains in the 1980s. One major disadvantage of this lack of genetic diversity is the universal susceptibility of mushroom crops worldwide to the same pathogens, including viruses. At the moment, control measures rely entirely on good crop hygiene to prevent cross-contamination and transmission of the viruses between crops.

A virus breaker

In the past, another *Agaricus* species, *A. bitorquis* was used as a 'virus breaker' strain as it was vegetatively incompatible with commercial *A. bisporus* strains and so virus transmission was halted when this species was grown. These days, *A. bitorquis* is not commercially acceptable; however, an *A. bisporus* culture that is incompatible with current commercial strains, and is commercially acceptable, could potentially work as a new virus breaker strain. There is a lack of knowledge of how *A. bisporus* responds to virus infection at a genomic and proteomic level, and so the main aim of this project was to characterise the vegetative interactions between a variety of genetically different strains, as well as their responses to viral infection. A modern mid-range hybrid, Strain D, and four others (Strains A, B, C and E) with different levels of vegetative incompatibility with Strain D, were used. At the same time a fluorescence *in situ* hybridisation (FISH) method was evaluated to visualise where viruses are located within the fungal mycelium to enhance our understanding of virus dynamics within the host.

FIGURE 1: Hyphal fusions between two compatible strains.

Interactions, compatibility and virus transmission

Fungi grow by means of fine filaments of cells called hyphae, which radiate outward, looking for substrate and nutrition. When the hyphae from two compatible cultures come into close contact with each other, they can fuse together by a phenomenon known as hyphal anastomosis. In our studies, such hyphal fusions occurred regularly between compatible cultures (**Figure 1**) but they were also shown to occur between incompatible strains, although at a lower level (O'Connor *et al.*, 2020a). This low level of hyphal anastomosis allowed transmission of the AbV16 virus from virus-infected culture MVX-1153 into the mycelium of all the other strains tested; thus, an *A. bisporus* virus breaker strain may be a challenge to develop. Virus transmission was also evident under cropping conditions where AbV16 was detected in all strains (**Figure 2**). However, only a few mushrooms of the less-compatible Strains A, B, C and E tested positive, indicating that although hyphal fusion and virus transmission had occurred, it was less effective than for Strain D (**Figure 2**). Proteomic analysis indicated that Strains C and E responded to virus infection differently, compared to the high stress response of Strain D, thus opening avenues to identify antiviral characteristics and defence responses among genetically different *A. bisporus* strains.

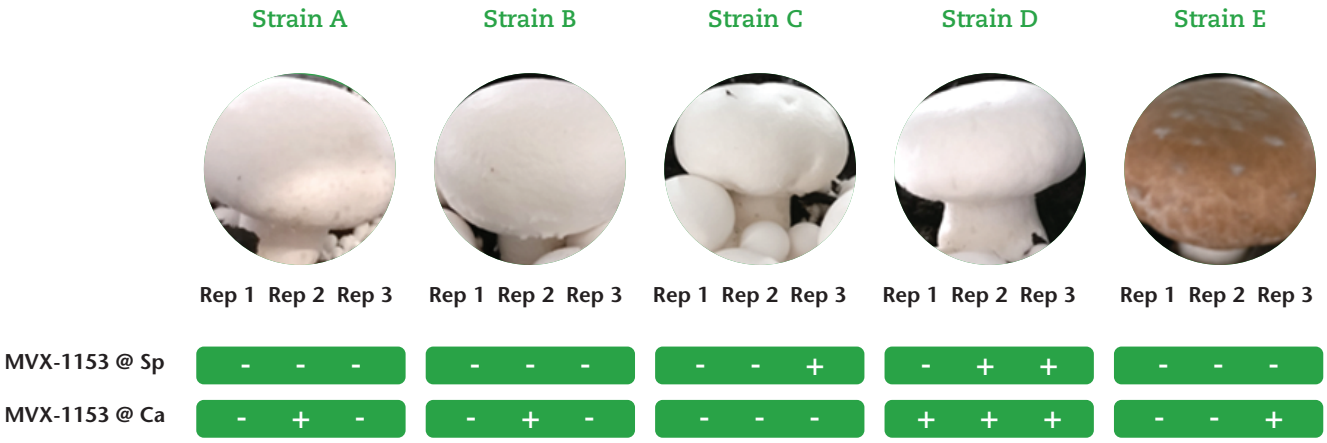

FIGURE 2: Detection of AbV16 (-/+) in crops following infection at spawning (@ Sp) or at casing (@ Ca).

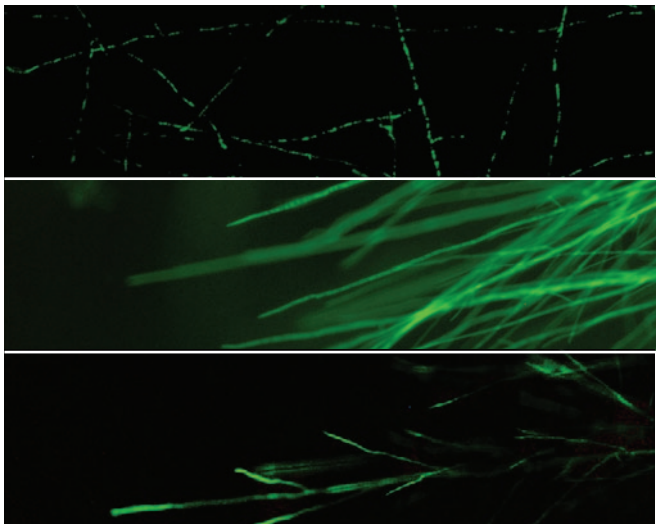

FIGURE 3: AbV16 virus detected in internal fungal hyphae (top); AbV6 virus in peripheral hyphae (middle); and, hyphal tips (bottom).

Virus visualisation

The FISH technique was used with excellent results to visualise where two viruses, AbV16 and AbV6, were located within the mycelium of infected cultures (O'Connor *et al.*, 2020b). Fluorescently labelled AbV16 showed high intensity and a compartmentalised distribution within the hyphal network, while the AbV6 virus was located more at the periphery of the culture and at hyphal tips (**Figure 3**), reinforcing the idea that virus transmission is likely during anastomosis events, even if only a small number occur.

Conclusion

These new insights into the different responses of *A. bisporus* strains to each other, and to virus infection, will enhance our understanding of potential barriers to viral transmission through vegetative incompatibility, as well as through antiviral activity, guiding future strain-breeding programmes.

References

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