

Ensuring the safety of mushrooms



TEAGASC research is looking at ways to control *Listeria monocytogenes* biofilm production and possible application of biocontrol agents in mushroom production environments.

Listeria monocytogenes is the causative agent of listeriosis in humans. Despite listeriosis being relatively rare, it is a major concern for the food industry, not only due to the severity of the symptoms and the very high hospitalisation and fatality rates (20-30%), but also because of the issues with product recalls/withdrawals. It poses a threat to all fresh fruits and vegetables, including mushrooms, due to its ubiquitous presence in the natural environment. There have been no reports of listeriosis due to the consumption of fresh cultivated mushrooms (*Agaricus bisporus*). Nonetheless, studies have shown that *L. monocytogenes* can be found in mushroom production facilities, which therefore poses a risk of product contamination. Across Europe, a number of *L. monocytogenes* associated recalls of mushroom products have occurred in recent years, and while none of these recalls were linked with causing listeriosis, they resulted in an economic and reputational loss for the industry. Thus, it is important to take proactive steps to maintain this industry's current reputation for food safety by pinpointing which areas of the mushroom production environment are of particular concern and by exploring novel biocontrol agents to provide enhanced assurance of product quality and safety.

Biofilms on industry-relevant surfaces

L. monocytogenes can survive under adverse conditions in different types of environments, including food production environments, in part due to its ability to form biofilms. Being in a biofilm state gives *L. monocytogenes* enhanced resistance to cleaning and decontamination procedures, and also allows it to adhere to different surfaces. A key objective of this Department of Agriculture, Food and the Marine (DAFM)-funded project was to determine the biofilm formation potential of *L. monocytogenes* strains, isolated from the mushroom production environment, on surfaces relevant to mushroom production. The biofilms were all

formed on 12 different surfaces using a bioreactor. As illustrated in **Figure 1**, *L. monocytogenes* strains were able to form biofilms on all surfaces tested. Most of the surfaces supported biofilms containing *L. monocytogenes* counts of Log_{10} 4-5 CFU/cm², while concrete and Nicotarp had counts of Log_{10} 6-7 CFU/cm². Copper was found to support significantly less biofilm. Concrete was a particular concern, as it makes up all the floor surfaces in production environments. A significant reduction of biofilm levels on concrete was observed when the concrete was painted with a concrete sealant.

Lactic acid bacteria as a biocontrol agent

A second aspect of this project was to investigate the potential utilisation of bacteriocin-producing bacteria that may be present in the mushroom production environment, as biocontrol agents to inhibit or control *L. monocytogenes*. Potential anti-listerial bacteria were screened from different types and phases of mushroom growth substrates from a number of production facilities. Isolates with anti-listerial activity were then identified and compared using whole genome sequencing, while the bacteriocins produced were identified using MALDI-TOF mass spectrometry. In this trial, all the isolates with anti-listerial activity were identified as *Lactococcus lactis* subsp. *lactis* and were all nisin Z producers.

Nisin is a commercially employed bacteriocin with a 'generally regarded as safe' (GRAS) status. Competitive exclusion activity of the mushroom production unit-derived *L. lactis* subsp. *lactis* was then tested on stainless steel coupons for three days at 25°C. Mixed culture biofilms of *L. monocytogenes* and *L. lactis* subsp. *lactis* resulted in a Log_{10} 4 CFU/cm² reduction in *L. monocytogenes* in comparison to a *L. monocytogenes* monospecies biofilm.

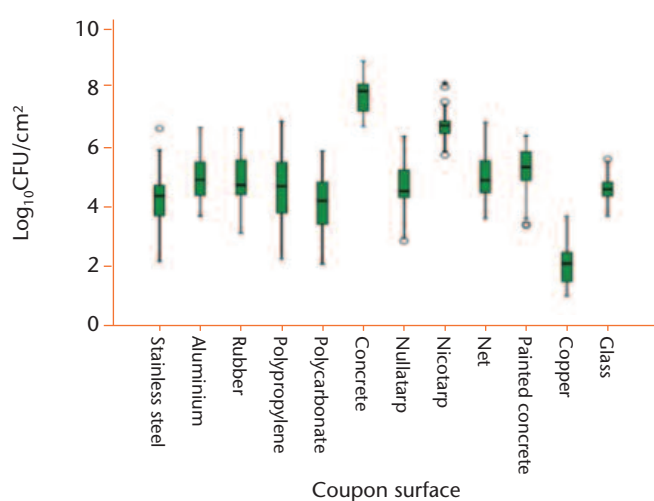


FIGURE 1: Boxplots of biofilm formation of seven *L. monocytogenes* strains after 72 hours on different surfaces.

Conclusions and further research

The results obtained from this project identified areas that may support greater levels of *L. monocytogenes* biofilm formation. Moreover, *L. lactis* subsp. *lactis* has been shown in this study to be naturally present in the mushroom production environment, perhaps providing a natural protection against *L. monocytogenes*, and has the potential to be used as a natural biocontrol agent. This application will be tested in the Teagasc pilot-scale mushroom production unit where the ability of this biocontrol agent to control *L. monocytogenes* will be put to the test during a normal crop production cycle, while concomitantly monitoring product quality and yield.

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