Grower Summary

Improved understanding and control of bacterial blotch and green mould in mushroom production

M065

Final report 2022

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Project leader:	Joana G. Vicente, Fera Science Ltd.
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Key staff:	Dr Joana G. Vicente (Fera Science Ltd); Dr Ralph Noble (Microbiotech Ltd); Professor George Salmond (University of Cambridge) And also: Andreja Dobrovin-Pennington (Microbiotech Ltd.), Dr Jessica Bergman, Josh Western (University of Cambridge); John Elphinstone, Ian Adams, Sam McGreig, Ann Barnes, Christopher Field, Ashleigh Elliott, Marco Benucci, Lynn Laurenson, Hannah Marsay, Adam Bryning, Ross Harland, Jennifer Cole, Brian Carter (Fera Science)
Location of project:	Fera Science Ltd, National Agri-food Innovation Campus, Sand Hutton, York YO41 1LZ Microbiotech Ltd, Marriott Building, Pershore Centre, Avonbank, Pershore, Worcestershire, WR10 3JP, UK Department of Biochemistry, University of Cambridge, Hopkins Building, Tennis Court Road, Cambridge CB2 1QW
Industry Representative:	Dr Phil Morley, APS Produce John Hall, Monaghan Mushrooms
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

GROWER SUMMARY

Headlines

- Several new blotch causing *Pseudomonas* species and green mould causing *Trichoderma aggressivum* f. *europaeum* isolates have been obtained from UK farm samples and identified
- New real time PCR assays can identify groups of blotch causing *Pseudomonas* that were not detected using previously developed assays targeting *P. tolaasii* and *'P. gingeri'*
- Bacterial and fungal populations have been sequenced and compared on samples collected from four farms; samples of casing and substrate had distinct populations; *Trichoderma* was detected at a high level in one farm and low level in two other farms
- The detection of *Trichoderma* spp. in farm samples was confirmed by qPCR and MinION sequencing
- Irrigation of casing with a non-pathogenic pseudomonad strain showed promising control levels of three blotch types in a pot bioassay and showed reduction of 40% of blotch on the first flush in a farm experiment
- Bacteriophages isolated from river water and mushroom farm samples have been characterised and shown to reduce blotch in a controlled bioassay

Background

Bacterial blotch is a mushroom disease that has been shown to be caused mainly by the bacterial species *Pseudomonas tolaasii*, *P. costantinii* and several groups of *'P. gingeri'* in the UK. This disease is considered to be the most important disease currently faced by the mushroom industry in the UK and elsewhere in Europe, causing losses that can frequently exceed 30% of production. Conditions that favour high yield are also favourable for disease development and transmission, and therefore there is a trade-off between maximising yield and maintaining health and quality of production. The development of tools that allow early detection of disease and understanding the possible sources of infection should be beneficial to the industry. In this project, we furthered our understanding of the communities of microorganisms involved in mushroom production and developed practical control measures that can reduce or eliminate spread of pathogenic pseudomonads without having a negative

impact on beneficial populations that are necessary for mushroom production. The tools developed have potential to be used across the supply chain to reduce losses and production costs and guarantee sustainable supply.

Although the number of outbreaks of compost green mould caused by *Trichoderma aggressivum* f. *europaeum* has been reduced through the implementation of sanitation measures, we confirmed that this disease still occurs in some farms causing significant losses. The American form of the pathogen (*Trichoderma aggressivum* f. *aggressivum*) so far has not been detected in the UK but constitutes an additional threat to mushroom production. Early detection of *Trichoderma* species has the potential to be used to inform control strategies and to monitor general farm hygiene.

This project follows on from project M 063 and the main aims are to detect, monitor and control blotch-causing pseudomonads and *Trichoderma* species whilst retaining populations of beneficial microorganisms in mushroom cultivation. In particular, the aims are to:

1. Enable sensitive detection in fresh substrates of all blotch-causing *Pseudomonas* species to determine if the analysis relates to the occurrence of blotch, thereby predicting disease risk
2. Determine the relative abundance of blotch-causing pseudomonads, *Trichoderma* species and other microorganisms in mushroom cropping substrates from different sources and in response to control treatments at different stages of commercial production
3. Estimate degree of control of blotch and/or green mould achieved by irrigating with antagonists, bacteriophages and ionic solutions
4. Make diagnostic tests available and disseminate the results to the mushroom industry

Summary

Blotch detection and control

Bacterial isolates obtained from mushrooms from several UK farms with symptoms of severe brown blotch, pitting and strong and mild ginger blotch were identified as *Pseudomonas tolaasii*, *P. costantinii* and several groups of *'P. gingeri'* respectively. A Pseudomonad isolated from a mushroom of the brown strain Heirloom with dark brown blotch symptoms was confirmed as being *P. tolaasii* following pathogenicity tests, qPCR and sequencing.

Comparison of whole genome sequences, showed that there are at least five different groups of isolates currently included in *'P. gingeri'* that can cause ginger blotch in UK farms; four

isolates that were not detected by previously developed qPCR assays belonged to three groups of *'P. gingeri'*. Pathogenicity (the ability to cause disease) in mushrooms was confirmed for a range of isolates in cap droplet inoculation tests and pot culture tests. A new method to test the pathogenicity in mushrooms grown in small pots enclosed in plastic bags has been developed and used successfully in experiments to test an antagonist that can reduce the level of disease seen in a crop.

New TaqMan assays have been developed in the current project based on recently obtained whole genome sequences, to detect groups of pathogenic *Pseudomonas* that were not detected by previously developed real-time assays that only targeted *P. tolaasii* and some groups of *'P. gingeri'* (project M 063).

The counts of background and pathogenic Pseudomonads can be increased by adding a compound to Luria-Bertani broth during incubation of casing extracts. This can help achieve detectable concentrations in samples containing low levels of the Pseudomonad populations.

Commercially available pseudomonads that are used to control pathogens or as growth promoters in other crops, including *Pseudomonas putida*, *P. fluorescens* and *P. chlororaphis*, did not reduce the incidence of blotch. Irrigation with ionic solutions did not consistently reduce the incidence of blotch in controlled small pot assays.

Non-pathogenic Pseudomonads from culture collections were also tested as potential antagonists to control blotch. Application of inoculum from a *'P. reactans'* isolate P7759 (a non-pathogenic isolate from mushrooms) to pots resulted in an increase in the number of healthy mushrooms compared with water treated pots, except for pots inoculated with *'P. gingeri'*; brown blotch caused by *P. tolaasii* was reduced by the application of P7759**.** In an on-farm experiment, application of P7759 inoculum resulted in a reduction in the number of blotched mushrooms that was not statistically significant.

Bacteriophages (viruses that can infect and destroy bacteria) that target most isolates of *P. tolaasii, P. costantinii* and *'P. gingeri'* were obtained and characterised. The application of bacteriophages resulted in significantly fewer blotched mushrooms in a pot culture bioassay, although there was no corresponding increase in the number of clean healthy mushrooms.

Green mould detection and control

Results of sequencing of two partial genes of strains of *Trichoderma* species from a culture collection hosted at Fera and from recent farm isolates, resulted in changes to the original culture designations. Cultures obtained from mushroom substrates show that *T. aggressivum* f. *europeum* was prevalent on two farms. PCR assays were selected for the detection of *Trichoderma* spp. at genus, species and subspecies level.

A potential antagonist, *Bacillus subtilis* syn. *B. amyloliquefaciens* AHDB 9849, was tested in pot experiments and was shown to be ineffective in suppressing green mould caused by *T. aggressivum* in compost.

Microbial communities

A study of microbial communities in cropping substrates obtained from four commercial farms, sampled at different cropping stages, was conducted to compare populations in healthy and diseased crops. Microbiome sequencing of bacterial and fungal communities showed differences between substrate and casing and some differences between farms. The methods used did not allow the identification of different *Pseudomonas* species, but successful detection of *Trichoderma* was achieved. A specific qPCR assay developed at Fera for *T. aggressivum* and MinION ITS sequencing detected these pathogens in mushroom casing at concentrations that did not produce visible green mould symptoms.

Financial Benefits

Although it is too early to state and calculate the financial benefits of this work, the development and selection of assays that can detect most blotch causing *Pseudomonas* and *Trichoderma* species can lead to financial benefits if used to make early decisions on disease management.

The identification of potential biocontrol agents including a *Pseudomonas* strain and bacteriophages might lead to significant financial benefits.

Action Points

- A range of diagnostic tests for *Pseudomonas* spp. causing blotch have been developed during this project to include most of the blotch causing pathogens identified in the UK; these tests, available at Fera Science (and planned to be published), are recommended for identification of *P. tolaasii*, *P. constantinii* and most groups of *'P. gingeri'*
- Pathogenicity of other species of *Pseudomonas* that might be present in UK farms should be further investigated (and further assays developed if necessary)
- A *Pseudomonas* sp. strain was shown to have the potential to reduce mushrooms with blotch and increase the number of healthy mushrooms. The application of this

strain as a commercial product should be further tested in different farms in order to assess the potential benefits of the treatments

- Further work is needed to test a range of bacteriophages, individually and/or in cocktails, as potential biocontrol agents and to develop a strategy for their use.
- Diagnostic tests for *Trichoderma* spp. including PCR tests for all *Trichoderma* spp. and a qPCR assay for T*. aggressivum,* the cause of green mould, are published and available (at Fera Science) and are recommended for detection of *Trichoderma* spp. that can be linked to issues in farm hygiene that should then be addressed
- Development of qPCR assays for *Trichoderma* at genus and subspecies level could be useful for quick assessment of farm hygiene
- Further studies involving microbiome sequencing are needed to characterise healthy and disease-linked microbe communities