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GROWER SUMMARY

Headline

- Of 40 fungal mushroom pathogen isolates retrieved from liquid nitrogen storage, 33 were successfully recultured and stored in water agar at Fera.
- Of 40 bacterial isolates (mushroom pathogens and initiation stimulators), all were successfully recultured and stored by -80ºC cryopreservation and by freeze drying (lyophilisation).

Background and expected deliverables

The liquid nitrogen supply of the mushroom pathogen culture collection at the University of Warwick was turned off in the summer of 2012. This aim of this work was to retrieve the most significant cultures and deposit them into low temperature storage systems at Fera, and check their viability and purity.

Summary of the project and main conclusions

Bacterial and fungal isolates were retrieved from storage in liquid nitrogen and cultured on agar plates. Of 40 fungal pathogen isolates retrieved from liquid nitrogen storage, 33 were successfully recultured and stored in water agar at Fera. The successfully retrieved cultures consisted of 18 *Verticillium fungicola* (or *V. malthousei*) isolates, nine *Trichoderma* species isolates, three *Gliocladium* isolates, two *Penicillium* isolates, one *Corticum* isolate and one *Cladobotryum* isolate. Of 40 bacterial isolates (mushroom pathogens and initiation stimulators), all were successfully recultured and stored by -80˚C cryopreservation and by freeze drying (lyophilisation). All of the bacterial isolates were originally labelled as *Pseudomonas* species, consisting of 21 un-named *Pseudomonas* species isolates, 7 *P. tolaasii*, 4 *P. agarici*, 4 *P. putidia*, 2 *P.* Reactans, 1 *P. syringae* and 1 *P. fragi* isolate.

Benefits to industry

Retrieval and storage of the bacterial and fungal pathogen cultures will be of value in future mushroom pathology research projects. The historical cultures will enable comparison with, for example, appearance of new fungicide resistant and/or virulent strains of pathogens in the mushroom industry. The retrieved cultures have named locations and dates of collection. Bacteria include strains of blotch and drippy gill pathogens and mushroom initiation stimulators (*Pseudomonas* spp.). Fungi include strains of green mold (*Trichoderma* spp), dry bubble (*Verticillium fungicola*), cobweb (*Cladobotryum* spp.) and compost smoky mould (*Penicillium* spp.).

SCIENCE SECTION

Introduction

The collection of mushroom pathogen cultures held in liquid nitrogen storage at Warwick University was assembled from the mid-1970s onwards, firstly at the Glasshouse Crops Research Institute (GCRI) and then at HRI Wellesbourne and Warwick HRI. The collection includes material that is of value to future mushroom research projects on bacterial (*Pseudomonas* spp.) and fungal (*Trichoderma*, *Verticillium*, *Cladobotryum*) pathogens and compost molds (*Penicillium* spp.). The majority of these isolates has been tested for pathogenicity in mushroom experiments and/or was obtained from disease outbreaks on the mushroom units or other mushrooms farms. This included large screening programmes for bacterial blotch and dry bubble diseases in the 1970s. In the event of future disease epidemics in the mushroom industry, the pathogens responsible can be compared and tested against selected isolates from the culture collection. This will determine, for example, whether the new and historical isolates are genetically different, and if new isolates are more virulent and/or resistant to control measures.

In view of the high running costs of the liquid nitrogen storage system (around £500 per month) the liquid nitrogen supply to the culture collection was turned off in the summer of 2012. The cultures therefore needed to be transferred to alternative low temperature storage systems at Fera.

Project objectives

(a) To retrieve bacterial and fungal pathogen cultures from liquid nitrogen storage at Warwick University on to plate cultures (EMR)

(b) To put cultures into low temperature storage facilities at Fera for future mushroom pathology research projects (Fera)

(c) To test viability and purity of cultures (Fera)

Materials and methods

Bacterial and fungal isolates were stored in sealed drinking straw type ampoules in liquid nitrogen (Elliott 1976). Forty isolates of bacteria and fungi were retrieved from the storage vessels. Ampoules were rapidly thawed in a water bath at 30˚C and the contents plated on to nutrient agar (bacteria) or potato dextrose agar with tetracycline antibiotic (fungi). Three replicate ampoules of each isolate were retrieved. The culture

plates were incubated at 20˚C for 7-21 days and then stored at 4˚C for up to 4 weeks until transfer to Fera.

Fungal cultures were transferred on to new agar plate cultures, examined for purity and stored in sterile distilled water at 4˚C.

Bacterial cultures were stored in -80˚C (cryopreservation) and by freeze drying (lyophilisation), retrieved and tested for purity.

Results

The culture nomenclature used in the original collection has been retained. In some cases, the nomenclature used has been superseded (e.g. *Verticillium fungicola* replaced *V. malthousei*). In other cases where cultures were originally identified from their morphology, molecular taxonomy techniques may result in different species names being used in the future. It is possible that some of the isolates labelled as *Trichoderma harzianum* or *Gliocladium* species are other *Trichoderma* species.

Retrieval of fungal and bacterial isolates

Of the 40 fungal isolates retrieved from liquid nitrogen, 33 were successfully recultured and stored in water agar at Fera (Table 1). The only species which could not be retrieved was *Pythium*, of which two isolates (*P. oligandrum* and *Pythium* sp. 291) failed to produce viable cultures. The successfully retrieved cultures consisted of 18 *Verticillium fungicola* (or *V. malthousei*) isolates, nine *Trichoderma* species isolates, three *Gliocladium* isolates, two *Penicillium* isolates, one *Corticum* isolate and one *Cladobotryum* isolate. Elliott & Challen (1981) obtained a retrieval success rate of 95% for mushroom strains that were stored in liquid nitrogen for 3-4 years. The retrieval success rate here was 83% for fungal strains that had been stored for up to 30 years.

Of the 40 bacterial isolates retrieved from liquid nitrogen (Table 2), all were successfully recultured and stored at Fera. All of the isolates were originally labelled as *Pseudomonas* species, consisting of 21 un-named *Pseudomonas* species isolates, seven *P. tolaasii*, four *P. agarici*, four *P. putidia*, two *P.* Reactans, one *P. syringae* and one *P. fragi* isolate.

Table 1. Fungal pathogen cultures retrieved and stored at Fera

Pathogen (as labelled)	Isolate/Collector	Date collected
Cladobotryum penicilloides	D.G.Gandy, GCRI	10/04/1980
Penicillium implicatum	GCRI 13	28/01/1981
Penicillium sp. II	1043D	?
Trichoderma atroviride	T43	?
Trichoderma harzianum	IMI 275950	26/07/1983
Trichoderma harzianum	IMI 284726	26/07/1983
Trichoderma harzianum	24651 (Th1)	?
Trichoderma harzianum	278 (Th1)	?
Trichoderma harzianum	T7	?
Trichoderma koningii	163	?
Trichoderma pseudokoningii	17	?
Trichoderma sp.	P.B. Flegg, GCRI	10/04/1980
Verticillium fungicola	T.R. Fermor, GCRI	14/10/1986
Verticillium fungicola	FRANSAC1	?
Verticillium fungicola	VP, GCRI	?
Verticillium fungicola	VK, GCRI	?
Verticillium fungicola	VG, GCRI	01/12/1992
Verticillium fungicola	CSAC 133	?
Verticillium fungicola	CSMI 161	?
Verticillium fungicola	T.R. Fermor 1	1995 to 2000
Verticillium fungicola	T.R. Fermor 2	1995 to 2000
Verticillium fungicola	T.R. Fermor 3	1995 to 2000
Verticillium fungicola	T.R. Fermor 4	1995 to 2000
Verticillium fungicola	T.R. Fermor 5	1995 to 2000
Verticillium fungicola	G3 M.P. Challen/D.G. Gandy	14/10/1986
Verticillium malthousei	D.G. Gandy	10/04/1980
Verticillium malthousei	D.G. Gandy	23/01/1981
Verticillium malthousei	D.G. Gandy	23/01/1981
Verticillium malthousei	GCRI 23	19/03/1981
Corticum roseum	GCRI 33	20/04/1982
Gliocladium roseum	DMS	22/03/1983
Gliocladium roseum	NM/NEJ	07/06/1985
Gliocladium roseum	22-1 SMA/JMW	14/01/1986

Table 2. Bacterial pathogen and mushroom initiation stimulator cultures transferred to Fera

Species (labelled)	Isolate/Collector	Effect	Date collected
Pseudomonas agarici	NCPBB 2289	Drippy Gill	?
Pseudomonas agarici	CH6	Drippy Gill	?
Pseudomonas agarici	NCPPB 2289	Drippy Gill	?
Pseudomonas agarici	NCPPB 2472	Drippy Gill	?
Pseudomonas fragi	Pf 1	?	?
Pseudomonas putida	T3/JF bac	?	?
Pseudomonas putida	WB1	?	?
Pseudomonas putida	AN 202	?	?
Pseudomonas putida	S. Lincoln, n12	Initiation	01/11/2001
Pseudomonas Reactans	Keith Johnstone	?	?
Pseudomonas Reactans	ATCC 14340	Blotch	?
Pseudomonas sp.	NCPPB 3149	?	?
Pseudomonas sp.	NCPPB 3146	?	?
Pseudomonas sp.	711A	?	?
Pseudomonas sp.	4A Lux	Initiation	?
Pseudomonas sp.	ATCC 51309	Blotch	?
Pseudomonas sp.	ATCC 15313	Blotch	?
Pseudomonas sp.	ATCC 51310	Blotch	?
Pseudomonas sp.	ATCC 51312	Blotch	?
Pseudomonas sp.	ATCC 33618	?	?
Pseudomonas sp.	ATCC 51314	?	?
Pseudomonas sp.	ATCC 51315	?	?
Pseudomonas sp.	ATCC 51311	?	?
Pseudomonas sp.	S. Lincoln, T2/5	Initiation	?
Pseudomonas sp.	S. Lincoln, T1/4	Initiation	?
Pseudomonas sp.	S. Lincoln, T3/2	Initiation	?
Pseudomonas sp.	S. Lincoln, T2/6	Initiation	?
Pseudomonas sp.	S. Lincoln, Mar-12	Initiation	?
Pseudomonas sp.	S. Lincoln, Mar +1	Initiation	?
Pseudomonas sp.	S. Lincoln, Mar-02	Initiation	?
Pseudomonas sp.	S. Lincoln, Mar +13	Initiation	?
Pseudomonas sp.	S. Lincoln, NSC4	Initiation	?
Pseudomonas tolaasii	NCPPB 2192	Blotch	?
Pseudomonas syringae	?	?	?
Pseudomonas tolaasii	T.R. Fermor screening 1	Blotch	18/04/1986
Pseudomonas tolaasii	T.R. Fermor screening 2	Blotch	18/04/1986
Pseudomonas tolaasii	T.R. Fermor screening 3	Blotch	18/04/1986
Pseudomonas tolaasii	T.R. Fermor screening 4	Blotch	28/01/1988
Pseudomonas tolaasii	T.R. Fermor screening 5	Blotch	28/01/1988
Pseudomonas tolaasii	T.R. Fermor screening 6	Blotch	28/01/1988

Conclusions

- Of 40 fungal mushroom pathogen isolates retrieved from liquid nitrogen storage, 33 were successfully recultured and stored in water agar at Fera.
- Of 40 bacterial isolates (mushroom pathogens and initiation stimulators) all were successfully recultured and stored by -80˚C (cryopreservation) and by freeze drying (lyophilisation).

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