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Pathogens and Other Fungi in Growing Media Constituents

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Abstract

With the increasing availability of composted and other organic alternatives to peat in the UK and continuing encouragement to use them, growing media manufacturers have begun to seek assurance that every substrate they use, including peat, is free of plant and human pathogens. This study was initiated to validate and exploit the use a state-of-the-art nucleic acid-based technique to investigate the diversity of fungal species in a range of substrates representing peat (13 samples), composted green waste (9), woodfibre (6), coir (6) and bark (4). In all twenty-nine different species of fungi were identified in the 38 substrates tested, of which the majority (*Aspergillus*, *Chaetomium*, *Mortierella*, *Mucor*, *Penicillium*, *Verticillium* and other species) were benign saprotrophic organisms. Beneficial *Trichoderma* species (*T. asperellum*, *T. harzianum* and *T. viride*) were present in nine samples, mostly peats. Only two of the samples contained fungi that might be regarded as a potential threat to plant health, *Fusarium oxypsorum* f.sp. *melonis*, a pathogen of melons, and a species of *Rhizoctonia* pathogenic to barley and lupins. *R. solani* was demonstrably absent in all and so was the clubroot organism, *Plasmodiophora brassicae*, whose presence was also rigorously investigated using a sensitive antibody and PCR-based technique. This study has shown that modern molecular techniques can be used to provide a comprehensive assessment of contamination of growing media constituents, particularly by fungi such as *F. oxysporum* and other commercially significant organisms such as the plant pathogen *P. brassicae*. As a result of this study the technique is now being offered commercially.

INTRODUCTION

Continuing pressure on UK growing media manufacturers to replace peat with sustainable materials has led them to seek reassurance that all organic substrates used are free of plant and human pathogens. Phytosanitary certification will require growing media to be tested for certain key organisms such as plant pathogenic fungi, bacteria and viruses.

This paper describes a study, sponsored by the UK Growing Media Association (GMA), managed by Paul Waller Consulting and carried out in association with the University of Exeter and Eco Diagnostics Ltd. Its aim was to validate and exploit the use of a state-of-the-art nucleic acid-based technique to investigate the diversity of fungal species in a range of substrates representing peat, composted green waste and soil, woodfibre, coir and bark.

MATERIALS AND METHODS

Thirty-eight unfertilized substrates were provided in confidence, including the materials most commonly used in UK growing media at the present time. These can be divided into five groups as shown in Table 1.

Each substrate sample was allocated an individual code by the project manager. Individual growing media manufacturers were advised of their own substrate codes and they dispatched samples directly to Exeter University identified only by these codes. This enabled strict confidentiality to be maintained and the samples to be tested blind.

A previously developed baiting procedure (Thornton et al., 2002) was used to recover live propagules of fungi from the substrates. These were then grown in axenic culture, from which representative DNA samples were prepared for identification of species by a polymerase chain reaction PCR-based method. Fungi were identified by analyzing the internally transcribed spacer (ITS) regions of the rRNA-encoding gene unit.

The data relating to all samples were returned to the project manager for collation. Individual suppliers were then advised of the results relating to the samples they had supplied and a general summary was made available to all GMA members.

RESULTS

Detailed results are given in Tables 2 and 3. Twenty-nine different species of fungi were identified in the 38 substrates tested, of which the majority (*Aspergillus*, *Chaetomium*, *Mortierella*, *Mucor*, *Penicillium*, *Verticillium* and other species) were benign saprotrophic organisms.

Beneficial *Trichoderma* species (*T. asperellum*, *T. harzianum* and *T. viride*) were present in nine samples, mostly peats. Only two of the samples contained fungi that might be regarded as a potential threat to plant health - *Fusarium oxypsorum* f.sp. *melonis*, a pathogen of melons, and a binucleate species of *Rhizoctonia* pathogenic to barley and lupins. *R. solani* was demonstrably absent in all.

In addition to the tests for fungi, the substrates were rigorously tested for the presence of the clubroot organism *Plasmodiophora brassicae* using a combination of host bioassays, and highly specific and sensitive antibody and PCR-based techniques. None of the substrates contained *P. brassicae*. The sensitivity and accuracy of the clubroot testing procedure was verified using artificial infestation of the growing media with *P. brassicae* resting spores. The procedure was able to detect as few as 10 resting spores per gram of medium.

CONCLUSIONS

None of the 38 substrates tested contained any significant plant pathogenic strains of fungi and a number, of mostly peat substrates, contained beneficial *Trichoderma* species. *Plasmodiophora brassicae* and *Rhizoctonia solani* were universally absent. The majority of species detected were benign saprotrophs.

This study has shown that modern molecular techniques can be used to provide a comprehensive assessment of contamination of growing media substrates, particularly by fungi such as *F. oxysporum* and other commercially significant organisms such as the plant pathogen *P. brassicae*.

As a result of this study the technique is now being offered commercially and the GMA members intend to repeat this exercise in 2006.

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Literature Cited

Thornton, C.R., Pitt, D., Wakley, G.E. and Talbot, N.J. 2002. Production of a monoclonal antibody specific to the genus *Trichoderma* and closely related fungi, and its use to detect *Trichoderma* spp. in naturally infested composts. Microbiology 148:1263–1279.

Tables

Table 1. Descripti

Group (Grp
1. Peat
2. Composted gr waste (CGW)
3. Wood-based materials
4. Coir
5. Bark

Tables

Table 1. Description of substrate samples tested.

Group (Grp.)	Number of samples	Description
1. Peat	13	Sphagnum peats from England, Scotland, N. Ireland, the Republic of Ireland and Estonia plus English sedge peat
2. Composted green waste (CGW)	9	CGW of various ages from many of the major CGW processors in the UK
3. Wood-based materials	6	Fibre materials derived from virgin or used wood and composted materials derived from woodwaste and forestry co-products
4. Coir	6	Pith from India and Sri Lanka
5. Bark	4	Matured/aged or composted bark derived from pine and/or other conifer species

Table 2. Number of positive results for pathogenic, beneficial and all fungus species in each substrate group.

Fungi	Grp. 1 Peat	Grp. 2 CGW	Grp. 3 Wood-based	Grp. 4 Coir	Grp. 5 Bark	Total
Pathogenic species						
Fusarium oxysporum f.sp. *melonis*	1	0	0	0	0	1
Plasmodiophora brassicae	0	0	0	0	0	0
Rhizoctonia solani	0	0	0	0	0	0
Rhizoctonia sp.	1	0	1	0	0	2
Total number of positive results	2	0	1	0	0	3
Mean number of positive results	0.2	0.0	0.2	0.0	0.0	0.1
Beneficial species						
Arthrobotrys amerospora	1	2	1	0	0	3
Trichoderma asperellum	0	0	0	0	1	2
Trichoderma harzianum	0	0	0	0	1	1
Trichoderma viride	4	0	0	2	0	6
Total number of positive results	5	2	1	2	2	12
Mean number of positive results	0.4	0.2	0.2	0.3	0.5	0.3
All species						
Total number of positive results	25	12	9	13	2	12
Mean number of positive results	1.9	1.3	1.5	1.2	0.5	1.3

Table 3. Number of positive results for saprotrophic fungus species in each substrate group.

Species	Grp. 1	Grp. 2	Grp. 3	Grp. 4	Grp. 5	Total

Table 3. Number of positive results for saprotrophic fungus species in each substrate group.

Species	Grp. 1 Peat	Grp. 2 CGW	Grp. 3 Wood-based	Grp. 4 Coir	Grp. 5 Bark	Total
Aspergillus clavatonanicus	0	0	0	0	1	1
Chaetomium sp.	1	0	0	0	0	1
Cladosporium oxysporum	0	0	1	0	0	1
Eupenicillium reticulisporum	0	0	0	1	0	1
Galactomyces geotrichum	2	2	2	3	0	9
Kondoa aeria	0	0	0	1	0	1
Mortierella alpina	2	1	0	0	0	3
Mortierella gamsii	0	1	0	0	0	1
Mucor circinelloides f. sp. *circinelloides*	1	2	0	2	2	7
Mucor hiemalis f.sp. *silvaticus*	1	0	0	0	0	1
Paecilomyces fumosoroseus	1	0	0	1	0	2
Penicillium atramentosum	2	0	0	0	0	2
Penicillium crustosum	0	1	0	0	0	1
Penicillium griseoroseum	0	1	0	0	0	1
Penicillium janthinellum	0	0	0	1	0	1
Penicillium melinii	0	0	1	0	0	1
Penicillium ochrochloron	2	0	0	0	0	2
Penicillium olsonii	1	0	0	1	0	2
Penicillium paneum	1	0	1	0	0	2
Penicillium velutinum	1	0	0	1	1	3
Thielavia hyalocarpa	0	2	1	0	0	3
Trichosporon porosum	1	0	0	0	0	1
Verticillium coccosporum	2	0	1	0	0	3
Total number of positive results	18	10	7	11	4	50
Mean number of positive results	1.4	1.1	1.2	1.8	1.0	1.3