FOREST OWNERS ASSOCIATION

Programme: Bioprotection for foliar diseases and disorders of radiata pine

Isolation of *Trichoderma* root endophytes
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EXECUTIVE SUMMARY

Foliar diseases and disorders are the largest cause of economic loss for the New Zealand forestry industry. In order to alleviate losses caused by existing diseases and also reduce potential impacts of new biosecurity threats, research is being conducted to establish a long-term symbiotic relationship between radiata pine and beneficial microbes.

For the selection of strains with potential for suppressing foliar disease in *Pinus radiata* in New Zealand plantations, endophytic *Trichoderma* cultures were isolated from exceptionally healthy plants representing a wide range of plant species at several different sites.

A total of 232 *Trichoderma* cultures were isolated from roots of more than 66 species in 49 plant families in this project. These were added to the Bio-Protection Research Centre (Lincoln University) culture collection along with over 500 previously isolated *Trichoderma* root endophytes. Most of these were then screened for growth promotion and disease suppression.

The best isolates are being evaluated in forestry plantation trials.

INTRODUCTION

Fungi in the genus *Trichoderma* have been widely studied as biocontrol agents for plant disease and for their ability to stimulate plant growth. *Trichoderma* species are capable of inhibiting plant disease through direct parasitism, antibiotic production and competitive effects, and some strains also have the ability to form symbiotic endophytic associations with roots and induce systemic disease resistance in their host plants (Harman *et al.* 2004; Hermosa *et al.* 2012). Isolation of endophytic *Trichoderrma* strains from surface-sterilised roots of exceptionally healthy plants therefore represents a promising approach for the discovery of effective *Trichoderma* isolates for use in forestry bioprotection. This approach can significantly streamline the selection process for useful biocontrol strains and has proven extremely successful in improving forestry production in South East Asia (Hill *et al.* 2010). For the selection of strains with potential for suppressing foliar disease in *Pinus radiata* in New Zealand plantations, endophytic *Trichoderma* cultures were isolated from healthy plants from a wide range of plant species at several different sites.

METHODS

Root samples were collected from a range of plant species at the following main locations: Christchurch Botanic Gardens; Biological Husbandry Unit, Lincoln University; Plant and Food Research Truffle demo block, Lincoln. Permission for collection of root samples was obtained from relevant managers of each site. At each site, only plants that appeared exceptionally healthy were selected for root collection. For each plant selected, a small quantity of roots were exposed and removed. Roots were transferred to labelled zip-lock bags and moistened with water to prevent dessication. Following collection, samples were refrigerated at 4°C for up to one week before processing.

For each sample, roots were washed thoroughly under running tap water to remove soil and other debris. Roots were then cut into approximately thirty 0.5-10mm sections representing different types of root morphology (e.g. primary, lateral, feeder roots, etc.) Working in a laminar flow cabinet, 25 root pieces were surface-sterilised by submersion in a deep-well petri dish containing ~50 ml of freshly prepared 1% Virkon for 10 minutes. Dishes were gently agitated every few minutes. After 10 minutes, root pieces were rinsed by transferring to a deep well dish containing ~50 ml of sterile reverse osmosis water and gently agitated. Using flamed forceps, root pieces were removed and placed onto sterile filter paper and briefly air-dried to remove excess water. Five surface-sterilised root pieces were placed onto each of five plates of LU-TSM (McLean *et al.* 2005) or MRB medium (malt extract 1.0%, yeast extract 0.1%, quintozene 0.02%, rose bengal 0.015%, agar 2%) amended with 100mg/L chloramphenicol. Plates were incubated at 22°C for 7-10 days. *Trichoderma* colonies were identified by cultural characteristics and/or microscopic morphology and subcultured to plates of MYE agar (malt extract 1.0%, yeast extract 0.1%, agar 2%). Isolates were maintained as stock cultures in 20% glycerol frozen at -80°C.

RESULTS

A total of 232 *Trichoderma* strains were isolated into pure culture from roots of more than 66 species in 49 plant families (Table 1, below). All cultures are stored in the Lincoln University culture collection together with over 500 additional *Trichoderma* cultures previously isolated from plant roots using the same methodology.

Table 1. *Trichoderma* isolations from plant roots in 2012-2013.

Collection#	No. of isolates	Host species or substrate	Host family	Collection locality
FCC 372-378	7	*Aesculus hippocastanum*	Sapindaceae	ChCh Botanic Gardens
FCC 379-382	4	*Hypericum leschenaultii*	Hypericaceae	ChCh Botanic Gardens
FCC 383-386	4	*Acca sellowiana*	Myrtaceae	ChCh Botanic Gardens
FCC 387-397	11	*Celtis australis*	Ulmaceae	ChCh Botanic Gardens
FCC 398-400	3	*Indigofera cytisoides*	Papilionaceae	ChCh Botanic Gardens
FCC 401-403	3	*Agave* sp.	Agavaceae	ChCh Botanic Gardens
FCC 404-408	5	*Buddleia* sp.	Scrophulariaceae	ChCh Botanic Gardens
FCC 409-411	3	*Kniphofia* sp.	Xanthorrhoeaceae	ChCh Botanic Gardens
FCC 412-413	2	*Juglans nigra*	Juglandaceae	ChCh Botanic Gardens
FCC 414-415	2	*Gunnera* sp.	Gunneraceae	ChCh Botanic Gardens
FCC 416-419	4	n.d.	Compositae	ChCh Botanic Gardens
FCC 420-423	4	n.d.	Pteridophyta	ChCh Botanic Gardens
FCC 426-429	4	*Hosta* sp.	Asparagaceae	ChCh Botanic Gardens
FCC 430-432	3	*Crocosmia crocosmiiflora*	Iridaceae	ChCh Botanic Gardens
FCC 433-434	2	*Juniperus* sp.	Cupressaceae	ChCh Botanic Gardens
FCC 435-438	4	n.d.	Leguminaceae	ChCh Botanic Gardens
FCC 439-440	2	*Polygonatum* sp.	Asparagaceae	ChCh Botanic Gardens
FCC 441-443	3	Solanum tuberosum	Solanaceae	Potato farm, Southbridge
FCC 497	1	*Agapanthus* sp.	Liliaceae	ChCh Botanic Gardens
FCC 498-500	3	*Echium vulgare*	Boraginaceae	ChCh Botanic Gardens
FCC 501	1	*Polygonum* sp.	Polygonaceae	ChCh Botanic Gardens
FCC 502-503	2	n.d.	Lythraceae	ChCh Botanic Gardens
FCC 504	1	*Allium fistulosum*	Amaryllidaceae	Private garden, Prebbleton
FCC 505-506	2	*Mentha* sp.	Lamiaceae	Private garden, Prebbleton
FCC 507	1	n.d.	Poaceae	Private garden, Prebbleton
FCC 508-511	4	*Pinus pinaster*	Pinaceae	PFR truffle block, Lincoln
FCC 512-518, 531-540	17	*Pinus radiata*	Pinaceae	PFR truffle block, Lincoln
FCC 519-521	3	*Pinus pinea*	Pinaceae	PFR truffle block, Lincoln
FCC 522-524	3	*Pseudopanax arboreus*	Araliaceae	PFR truffle block, Lincoln
FCC 525-530	6	*Corylus avellana*	Betulaceae	PFR truffle block, Lincoln
FCC 531-532	2	*Malus domestica*	Rosaceae	BHU, Lincoln University
FCC 533-534	2	*Phormium tenax*	Xanthorrhoeaceae	BHU, Lincoln University
FCC 535-537	3	*Trifolium* sp.	Fabaceae	BHU, Lincoln University
FCC 538-540	3	*Vinca minor*	Apocynaceae	BHU, Lincoln University
FCC 541-542	2	*Poaceae*	Salicaceae	BHU, Lincoln University
FCC 543-544	2	*Populus* sp.	Salicaceae	BHU, Lincoln University

Table 1. Continued

Collection#	No. of isolates	Host species or substrate	Host family	Collection locality
FCC 545-549	5	*Quercus robur*	Fagaceae	BHU, Lincoln University
FCC 550-551	2	*Acer* sp.	Aceraceae	BHU, Lincoln University
FCC 552-553	2	n.d.	Poaceae	BHU, Lincoln University
FCC 554-555	2	*Galium aparine*	Rubiaceae	BHU, Lincoln University
FCC 556-557	2	*Anthriscus sylvestris*	Apiaceae	BHU, Lincoln University
FCC 558-559	2	*Rubus* sp.	Rosaceae	BHU, Lincoln University
FCC 560	1	*Symphytum* sp.	Boraginaceae	BHU, Lincoln University
FCC 561-563	3	*Larix* sp.	Pinaceae	BHU, Lincoln University
FCC 564-566	3	*Taraxacum officinalis*	Asteraceae	BHU, Lincoln University
FCC 567-569	3	*Pyrus* sp.	Rosaceae	BHU, Lincoln University
FCC 570-571	2	*Avena sativa*	Poaceae	BHU, Lincoln University
FCC 572-573	2	*Brassica napus*	Brassicaceae	BHU, Lincoln University
FCC 574-575	2	*Cytisus proliferus*	Fabaceae	BHU, Lincoln University
FCC 576-577	2	*Poaceae*	Poaceae	BHU, Lincoln University
FCC 578-580	3	*Brassicaceae*	Brassicaceae	BHU, Lincoln University
FCC 581-582	2	*Capsella bursa-pastoris*	Brassicaceae	BHU, Lincoln University
FCC 583-587	5	*Plantago* sp.	Plantaginaceae	BHU, Lincoln University
FCC 588-590	3	*Poaceae*	Poaceae	BHU, Lincoln University
FCC 591-593	3	*Dactylis glomerata*	Poaceae	BHU, Lincoln University
FCC 594-598	5	*Iris* sp.	Iridaceae	ChCh Botanic Gardens
FCC 599	1	*Broussonetia papyrifera*	Moraceae	ChCh Botanic Gardens
FCC 602-604	3	*Helleborus* sp.	Ranunculaceae	ChCh Botanic Gardens
FCC 605-609	5	*Rhododendron* sp.	Ericaceae	ChCh Botanic Gardens
FCC 610-613	4	*Syringa meyeri*	Oleaceae	ChCh Botanic Gardens
FCC 614-616	3	*Aquilegia* sp.	Ranunculaceae	ChCh Botanic Gardens
FCC 617-623	6	*Cistus* sp.	Cistaceae	ChCh Botanic Gardens
FCC 624-625	2	n.d.	Poaceae	ChCh Botanic Gardens
FCC 626-627	2	*Sophora* sp.	Fabaceae	ChCh Botanic Gardens
FCC 628	1	*Hebe* sp.	Scrophulariaceae	ChCh Botanic Gardens
FCC 629-631	3	*Podocarpus totara*	Podocarpaceae	ChCh Botanic Gardens
FCC 632-635	4	*Coprosma* sp.	Rubiaceae	ChCh Botanic Gardens
FCC 636-638	3	*Clianthus* sp.	Fabaceae	ChCh Botanic Gardens
FCC 639-641	3	*Colletia cruciata*	Rhamnaceae	ChCh Botanic Gardens
FCC 642	1	Unidentified fern sp.	n.d.	ChCh Botanic Gardens
FCC 643	1	*Corokia cotoneaster*	Argophyllaceae	ChCh Botanic Gardens
FCC 644-645	2	*Festuca glauca*	Poaceae	ChCh Botanic Gardens
FCC 646	1	*Libertia ixodes*	Iridaceae	ChCh Botanic Gardens
FCC 647-650	4	*Pittosporum* sp.	Pittosporaceae	ChCh Botanic Gardens
FCC 651	1	*Macropiper excelsum*	Piperaceae	ChCh Botanic Gardens

n.d. = not determined

CONCLUSIONS

A total of 232 *Trichoderma* cultures were isolated from roots of more than 66 species in 49 plant families in this project. These were added to the Bio-Protection Research Centre (Lincoln University) culture collection along with over 500 previously isolated *Trichoderma* root endophytes. Most of these were then screened for growth promotion and disease suppression.

Based on nursery and laboratory trials, the best isolates are being evaluated for growth promotion and foliar disease suppression in forestry plantation trials throughout New Zealand.

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