Bioprotection - enhancing growth and health Research Programme

Bio-Protection
Bioprotection science for New Zealand

Isolation and characterisation of fungal partners in the *Pinus radiata* root microbiome

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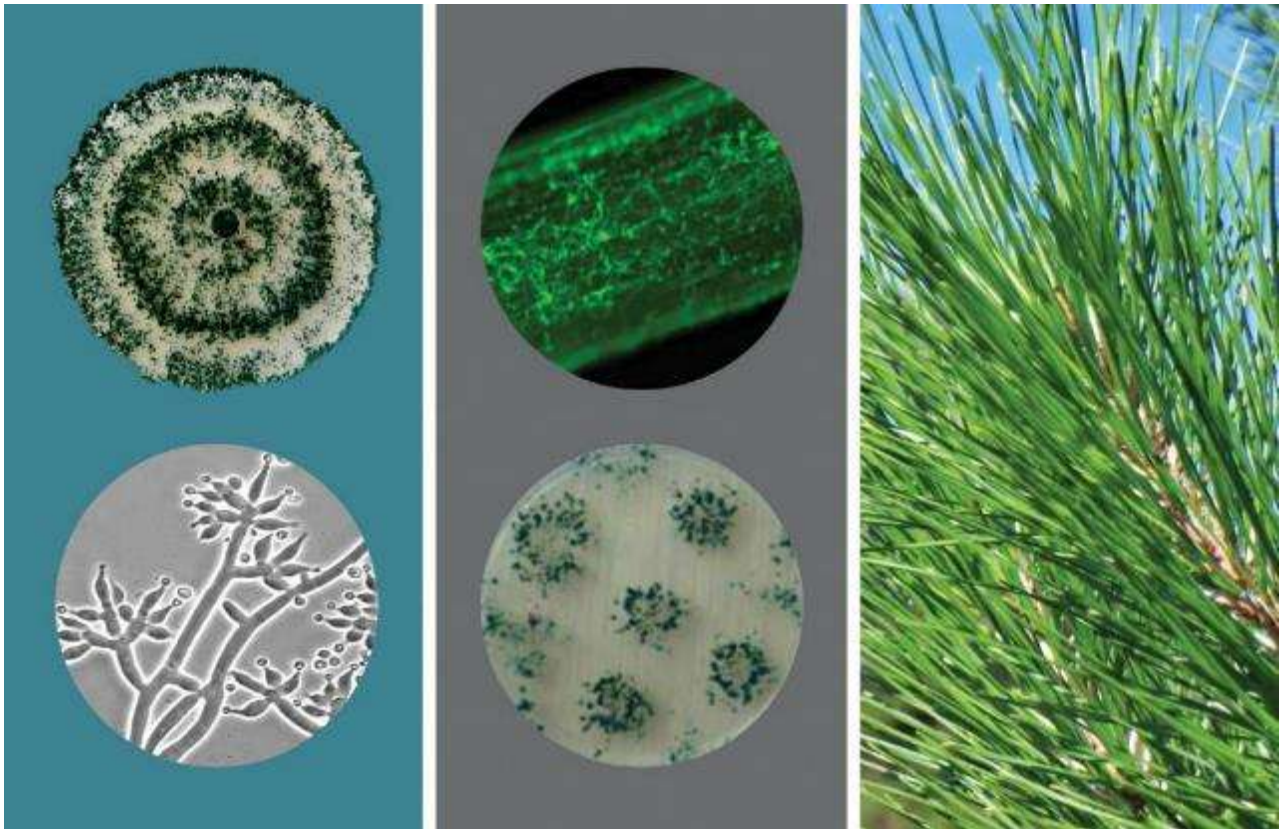

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Task 5.1

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EXECUTIVE SUMMARY

The main goal of the *Bioprotection for foliar diseases and disorders of radiata pine* programme is to induce systemic resistance against foliar diseases through the use of beneficial endophytes and elicitors. As part of this project, a number of plantation trials have been established at sites around New Zealand to examine the effects of specific endophytic *Trichoderma* strains on *Pinus radiata* growth and disease resistance. A major question arising from this work is whether the applied strains are able to persist under field conditions to provide long-term growth benefits and protection from disease. Persistence of these strains may be influenced by their interaction with other endophytic microbes naturally present in the plant roots. Characterisation of the background fungal endophyte microbiome in plantations will provide important prerequisite information for future examination of the effectiveness of the applied *Trichoderma* strains and possibly identify other species that could improve pine health.

This report describes results from a study of endophytic root fungi collected from *P. radiata* trees in nine New Zealand plantations throughout the North Island and Nelson and three small lots in Canterbury. Additional information for mature seedlings grown in greenhouse and nursery conditions was also included. Roots were surface-sterilised and fungal isolates were cultured using two culturing vessel methods. Isolates were identified using specific polymerase chain reaction (PCR) primers and direct sequencing to determine the background diversity of fungal species that are naturally present in plantations.

Key Results

- The isolate culturing method of agar-filled tissue culture plates was novel and allowed convenient and efficient processing of root samples and enabled the separation of fast-growing species from slow-growing species.

- Eighty-nine endophytic fungal isolates were identified through direct sequencing from *P. radiata* root samples collected in nine New Zealand forest plantations. They represented 26 genera and 57 species. In addition, 44 endophytic fungal isolates, representing 16 genera and 21 species, were identified from roots for the 8-month and 2-year-old *P. radiata* seedlings grown in greenhouse/nursery conditions. A total of 34 genera and 69 species were identified in the three studies combined.

- The genera with the most species diversity in the identified isolates were *Aspergillus, Fusarium, Ilyonectria, Penicillium* and *Trichoderma,* while the most common genera that grew on isolation plates *were Absidia, Cladophialophora, Fusarium, Mucor, Penicillium* and *Phialocephala* species.

- Ten *Trichoderma* species (*T. asperellum, T. atroviride, T. crassum, T. gamsii, T. hamatum, T. harzianum, T. koningii, T. koningiopsis, T. spirale* and *T. tomentosum*) were isolated from plantation sites in this study and this has contributed to the characterisation of the background population of *Trichoderma* in New Zealand *P. radiata* plantation systems.

INTRODUCTION

Endophytic *Trichoderma* fungi show great potential for improving health of forest trees. The main goal of the *Bioprotection for foliar diseases and disorders of radiata pine* research programme is to induce systemic resistance against foliar diseases through the use of beneficial endophytes and elicitors. Initial research in this programme isolated a large number of *Trichoderma* strains which were screened for the ability to promote growth and suppress disease in laboratory and nursery trials. The most effective isolates were applied as nursery seed treatments and form the basis for planation trials at sites around New Zealand.

Previous research focus has been on endophytic *Trichoderma* species associated with *P. radiata* (Cummings and Hill, 2016) where 16 species were identified through DNA sequence data. However, a wide range of fungal species from various genera may also internally colonise *P. radiata* roots including species which may promote growth, solubilise nutrients and provide protection from invertebrate pests. An important question about deployment of these *Trichoderma* isolates in the Bioprotection research programme is how resident fungal species present in the root may inhibit or otherwise interact with the applied *Trichoderma* isolates, potentially affecting the long-term bioprotection in the plantation.

There is limited information on the fungal microbiome in *P. radiata* roots, particularly in New Zealand plantation systems. The aim of Task 5.1 was to isolate fungal cultures from surface-sterilised roots collected from healthy plants at several New Zealand plantations and small lot sites to characterise the fungal microbes that occur naturally in forest plantations. Additional information for mature seedlings grown in greenhouse and containerised nursery experiments was also included. DNA analyses of partial *tef1* and ITS gene sequences of selected cultures was used for reliable, unambiguous identifications to species level for *Trichoderma* and other fungal genera, respectively. A second objective of Task 5.1 was to develop a novel culturing method for efficient processing of a large number of root pieces.

METHODS

Sample collection and fungi isolation

Roots were collected from four to eight healthy *P. radiata* trees in each of nine forest plantations and three small lot sites with collection details listed in Table 1. Further information for this report was obtained for roots from eight untreated 8-month old (Whelan and Hill, 2017) and 35 untreated 2-year-old (Brookes, 2014) *P. radiata* seedlings grown in greenhouse and containerised nursery conditions.

Table 1: Root sample collection details and number of isolates recovered in this study

Region	Location	Name of Forest Company and Plantation Name	Collection Locality Code	No. of root pieces plated on agar plates	No. of isolates sub-cultured	No. of isolates identified using sequence data
Northland	Whangarei	Hancock Timber Resource Group, Pipiwai	P	192	58	17
Northland	Kaikohe	Hancock Timber Resource Group, Otaenga	O	216	52	27
Nelson	Kohatu	Nelson Forests Ltd, Golden Downs (Kohatu block)	Ko	72	15	5
Nelson	Kikiwa	Nelson Forests Ltd, Golden Downs (Kings Ridge block)	KR	72	15	3
Gisborne	Inland Tokomaru Bay	Ernslaw One Ltd, Waiau	W	168	35	16
Gisborne	Inland Whatatutu	Ernslaw One Ltd, Waipaoa	Wp	111	12	5
Manawatu-Whanganui	Ohakune	Ernslaw One Ltd, Karioi	Ka	100	12	3
Manawatu-Whanganui	Whanganui	Ernslaw One Ltd Harakeke	H	96	2	2
Bay of Plenty	Kawerau	Hancock Timber Resource Group, Tawe	T	60	14	7
Canterbury	Banks Peninsula	Small lot, Kaituna Valley	KV	15	4	0
Canterbury	Banks Peninsula	Small lot, Motukarara	M	15	3	2
Canterbury	Mid-Canterbury	Small lot, Rakaia	R	15	7	2
TOTAL				1132	229	89
Canterbury	Lincoln	Greenhouse/nursery experiment, Lincoln University (Whelan and Hill, 2017)	GA	30	6	3
Canterbury	Lincoln	Greenhouse/nursery experiment, Lincoln University (Brookes, 2014)	GB	175	90	42
TOTAL				205	96	45

The plantation roots sampled were generally small feeder roots within the top 300 mm from ground level. Roots were moistened and stored in zip-locked bags at 4°C before processing. For fungal isolation, roots were washed thoroughly in running tap water and cut into pieces of approximately 15-20 mm lengths. Root pieces were surface-sterilised

under aseptic conditions by transferring through a series of petri dishes containing the following solutions and for the following soak times:

1) Triton X-100 (0.01%) for 3 minutes
2) ethanol (70%) for 1 minute
3) sodium hypochlorite (4%) for 3 minutes
4) ethanol (70%) for 30 seconds
5) sterile distilled water (three washes for 1 minute each).

Root pieces were gently agitated in each dish and solutions were discarded every three or four samples. Root pieces were placed on sterile paper to dry for 10 minutes and the ends of the root pieces were cut off on a chopping board and discarded. Root pieces were then cut into approximately 5-10 mm segments and placed onto MYE (malt extract 1.0%, yeast extract 0.2%, agar 2%) agar amended with antibiotics chlortetracyline hydrochloride (50 mg/L) and streptomycin sulphate (250 mg/L) in either tissue culture plates (1 mL agar per well; JET BIOFIL; Figure 1) or petri dishes (30 mL per plate; Figure 2). The isolation media and technique used in this study targeted root endophytic species that were not ectomycorrhizal. The isolation of ectomycorrhizal fungi associated with *P. radiata* (e.g. *Rhizopogon, Thelephora, Amanita, Wilcoxina, Suillus* sp.) require specialised, chemically defined media and techniques and was not attempted in this study.

Sterilisation of root pieces was confirmed by placing the following water or root samples, every four tree samples, onto MYE plates amended with antibiotics:

1) 100 µL water from the first and third water wash
2) water scraped from the chopping board
3) rolled root pieces.

A total of 60 sterilisation plates were tested by incubating under laboratory conditions (20-22°C) with ambient light and the presence or absence of fungal colonies was determined.

Plates with roots were incubated under laboratory conditions (20-22°C) with ambient light for 3-15 days. Fungal colonies were sub-cultured to plates of MYE agar (malt extract 1.0%, yeast extract 0.1%, agar 2%) and incubated under laboratory conditions (20-22°C) with ambient light for 3-10 days. Colonies selected for sub-culturing and DNA identification were chosen based on different visual morphological characteristics with the aim of capturing as much species diversity as possible. Sub-culturing was primarily from the Northland and Gisborne samples (Table 1). As new samples were received from different regions, progressively fewer colonies were selected for sub-culturing due to morphological similarity to previously colonies. Sub-cultures visually identified as *Trichoderma* were separated from other fungal samples due to the specificity of primers used in DNA extraction. For some samples, non-sequenced isolates were identified based on similar colony morphology. Samples of sub-cultures were stored in 2 mL cryovials at –80°C in the Bio-Protection Research Centre (Lincoln University, New Zealand) culture collection.

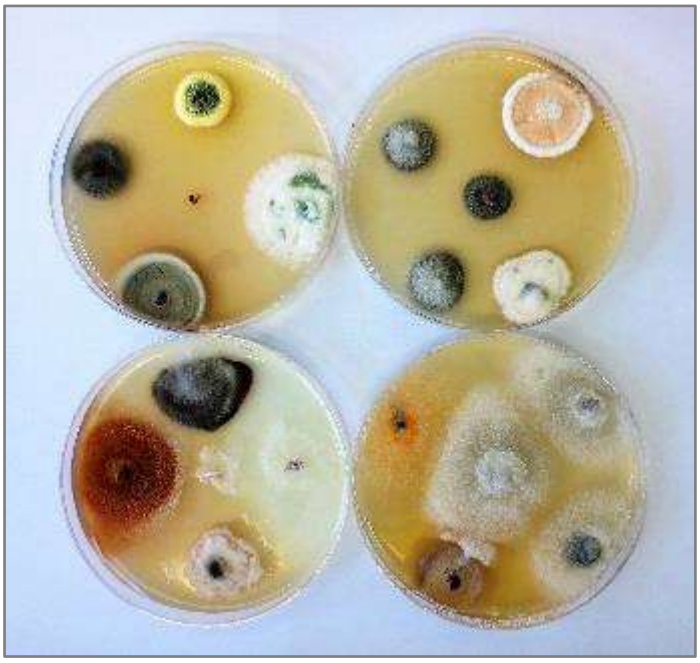

Figure 1: Seven-day old fungal colonies grown from pieces of surface-sterilised plantation *P. radiata* root plated on MYE agar, amended with antibiotics, in petri dishes.

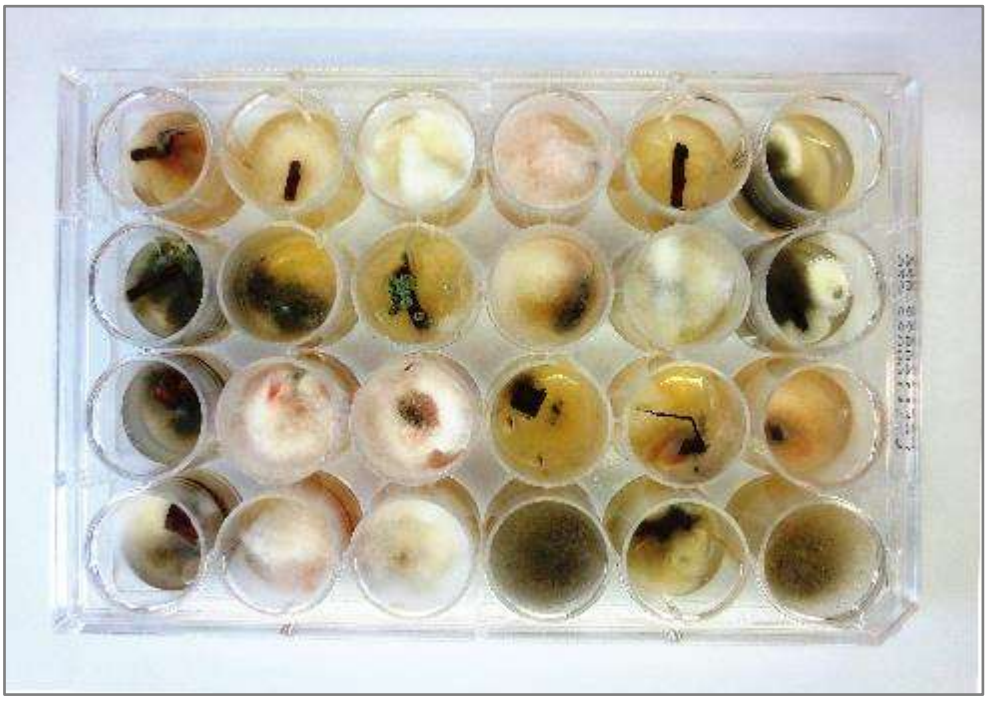

Figure 2: Seven-day old fungal colonies grown from single pieces of surface-sterilised plantation *P. radiata* roots plated on MYE agar, amended with antibiotics, in tissue culture plates.

DNA Extraction

Genomic DNA was extracted from vegetative mycelium using a Chelex 100 buffer method. Approximately 50 mg of 3-day-old mycelium was aseptically harvested from pure cultures and placed into sterile 1.7 mL Eppendorf tubes. Chelex 100 (0.5 mL, 5%, Bio-Rad Laboratories) was added to each tube and mycelium was ground with a sterile pestle. Tubes were placed in boiling water for 12 minutes then centrifuged at 13000 rpm for 30 minutes. The supernatant containing the DNA extract was pipetted into new 1.7 mL Eppendorf tubes and stored at 4°C.

Identification of Isolates using DNA Sequencing

Sequence data from the translation elongation factor 1α (*tef1)* gene is generally used for routine identification of *Trichoderma* species and can be used to differentiate all known *Trichoderma* species (Bissett *et al.,* 2015). For each *Trichoderma* isolate an approximately 900 base pair (bp) fragment from the 5' end of the *tef*1α gene was amplified using the primer pair tef85f (AGGACAAGACTCACATCAACG) and tef954R (AGTACCAGTGATCATGTTCTTG; Shoukouhi and Bissett, 2009). Sequence data from the internal transcribed spacer (ITS) region of the ribosomal RNA can be used to routinely identify diverse taxonomic groups of fungi (Toju *et al*., 2012). For isolates other than *Trichoderma*, an approximately 900 bp fragment from the 5'-3' end of the ITS gene was amplified using the primer pair ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (White et. al., 1990).

The quality of the DNA extracts was confirmed by conventional PCR of the *tef1* region with primers tef85f and tef954r for *Trichoderma* samples, and the ITS region with primers ITS4 and ITS5 for other fungal genera.

All PCR amplifications were performed in a total reaction volume of 25 µL, including 2.5 µL of 10x reaction buffer + 2.5 mM $MgCl_2$, 200 µM dNTPs, 400 µM of each primer, 0.5 µL Purified BSA (BioLabs, Inc.), 2.5 µL FastStart Taq DNA polymerase (Roche Applied Science) and 2 µL undiluted template DNA. PCR reactions were initiated with a DNA denaturing step for 5 minutes at 95°C; followed by 40 cycles each of 45 seconds at 95°C, 45 seconds at 56°C (for *Trichoderma* samples) or 57°C (for other fungal samples) and 2 minutes at 72°C; with a final extension step of 7 minutes for 72°C. A negative control (water used in PCR amplification) was included in each assay.

PCR products were visualised by staining with RedSafe (iNtRON Biotechnology) following electrophoresis of 6 µL of each product in 1.0% agarose gels (Figure 3). PCR products were then purified using a commercial cleanup kit (Agencourt CleanSEQ) and sequenced in the forward direction using the internal sequencing primer tef85f in an Applied Biosystems 3103xl Genetic Analyzer at the Bio-Protection Research Centre DNA Sequencing Facility (Lincoln University). Consensus sequences were assembled from forward sequencing chromatograms using ChromasPro v. 1.7.6 (https://technelysium.com.au/wp/chromaspro). Sequences were then searched in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee) to identify species affiliations.

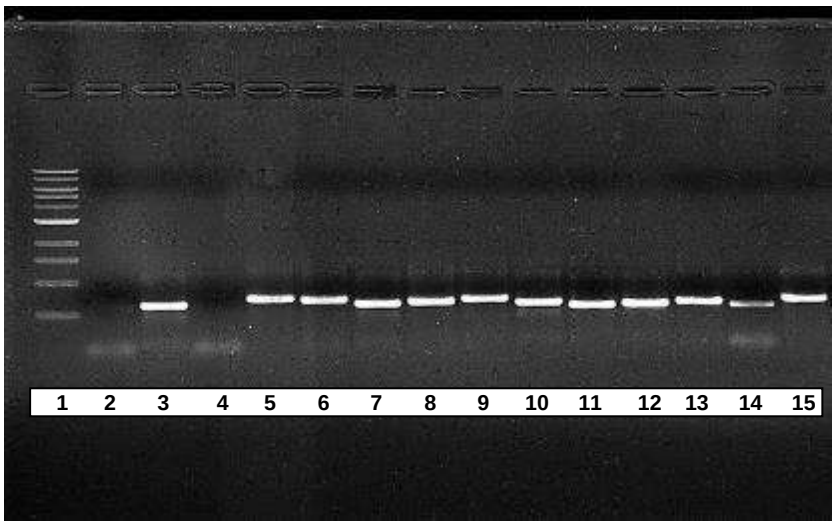

Figure 3: Example of PCR amplification, based on the chelex based method, of the ITS region in various endophytic fungal species in *P. radiata* roots (lane 1, DNA 1kb ladder; lane 2, negative water control; lane 3, *Penicillium pancosmium* or *ubiquetum (*25sub); lane 4, not sequenced (26), lane 5, *Gongronella butleri* (26sub); lane 6, *Umbelopsis changbaiensis* (41); lane 7, *Aspergillus parvulu* (42); lane 8, *Mortierella* sp. (43); lane 9, *Absidia glauca* (44); lane 10, *As. cervinus* (45); lane 11, *Ilyonectria rufa* (46); lane 12, *Rhizoscyphus* sp. (50); lane 13, *Resinicium bicolor* (51); lane 14, *Fusarium avenaceum* (52) and lane 15, *M. alpine* (53)).

RESULTS AND DISCUSSION

In total, 1110 fungal colonies grew out of the root pieces plated. Twenty percent of isolates (229), selected for being morphologically distinct, were plated to fresh media (examples shown in Appendix A). A total of 89 endophytic fungal isolates were identified through direct sequencing from *P. radiata* root samples collected in nine New Zealand forest plantations (Table 2), representing 26 genera and 57 species. Forty-four endophytic fungal isolates, representing 16 genera and 21 species, were identified in roots of the 8-month and 2-year old *P. radiata* seedlings grown in greenhouse/nursery conditions. A total of 34 genera and 69 species were identified in the three studies combined.

The genera with the most diversity in the identified isolates were *Aspergillus, Fusarium, Ilyonectria, Penicillium* and *Trichoderma* (Table 2), partly due to showing strong morphological variation, while the most common genera that grew on isolation plates were *Absidia, Cladophialophora, Fusarium, Mucor, Penicillium* and *Phialocephala* species (data not presented). Only 1.9% of root pieces did not produce endophyte colonies and may have contained no culturable fungal endophytes or the sterilisation treatment may have been too damaging to the tissue.

Ten *Trichoderma* species were isolated from plantation sites in this study and contribute to the characterisation of the background population of *Trichoderma* in New Zealand *P. radiata* plantation systems. Four species (*T. asperellum, T. gamsii, T. koningii* and *T. koningiopsis*) were in addition to those found in another *P. radiata* plantation *Trichoderma* study (Cummings and Hill, 2016), but these species were documented in an extensive survey of *Trichoderma* species in New Zealand (Braithwaite *et al.,* 2017) so are not considered to be novel. The impact of these natural *Trichoderma* species on the endophytic development of the applied *Trichoderma* strains is unknown.

Table 2: Fungi identification of plantation *P. radiata* root isolates

Isolate Code	Collection Locality Code [a]	Identification [b]	Comments
110 and 111, 44	Ka, Ko	Absidia glauca	
28	P	*Ab. psychrophilia*	
45	KR	*Aspergillus cervinus*	
11	P	*As. niger*	
42	KR	*As. parvulu*	
11sub	P	*As. welwitschiae*	
63, 64	W	*Cadophora orchidicola*	Synonym = *Leptodontidium orchidicolav*
96	M	*Chaetomium funicola*	
16sub	P	*Cladophialophora chaetospira*	Saprophytic endophyte in plant litter
40R	Ko	*Coniochaeta mutabilis*	Genus includes tree pathogens
122	O	*Corticiaceae* sp.	
7sub	P	*Cunninghamella elegans*	
54 and 67, 58	W, Wp	*Epicoccum nigrum*	
93	M	*Eurotiales* sp	
69R	W	*Fusarium acuminatum*	
52, 59	W	*F. avenaceum*	
61R	W	*F. lateritium* or *avenaceum*	
5	O	*F. oxysporum*	
18a, 26sub	P	*Gongronella butleri*	
14sub, 17sub	P	*Hyaloscyphaceae* sp.	Saprophytic fungi of dead wood and other plant matter
55 and 57, 60	W, Wp	*Ilyonectria cyclaminicola*	Genus often pathogenetic
104	Ka	*I. destructans*	
6	O	*I. radicicola*	
46	KR	*I. rufa*	
43	Ko	*Mortierella* sp.	
53	W	*M. alpine*	
19a, 109	O	*Mucor fuscus*	
68, 70R	W	*Mu. hiemalis*	
98	H	*Mu. moelleri*	
9, 10sub	O	*Penicillium amaliae*	
1aR	O	*P. bilaiae*	
23sub	O	*P. canescens*	
12	P	*P. citreonigrum*	
2	O	*P. glabrum*	
1, 9a	O	*P. lilacinoechinulatum* or *bilaiae*	
4d	P	*P. miczynski*	
66	W	*P. montanese*	
106	R	*P. murcianum*	
25sub	P	*P. pancosmium* or *ubiquetum*	
4a	P	*P pasqualense* or *restrictum*	
3sub	O	*P. sanguifluum*	
8a	O	*P. spinulosum*	

10	O	*P. thomaii*	
27	O	*Pestalotiopsis disseminata*	Opportunistic fungi; also a pathogen in banana
97	T	*Pezizales* sp.	
65	W	*Phacidiopycnis washingtonensis*	Described as a post-harvest fruit disease
62, 100	W, T	*Phialocephala fortinii*	Described as beneficial to tree growth
51	Wp	*Resinicium bicolor*	Pathogen of Douglas fir
50	Wp	*Rhizoscyphus* sp.	Some species are endophytic
20	P	*Talaromyces acaricola*	Some species are endophytic and described as potential biocontrol agents
13sub	P	*Ta. proteolyticus*	
123	O	*Trichoderma asperellum*	
34TB	O	*T. atroviride*	
80	T	*T. crassum*	
37TC	P	*T. gamsii*	
22, 22R, 32Asub, 36Asub	O	*T. hamatum*	
81, 125, 126, 127	T	*T. harzianum*	
30T, 33AR	O, P	*T. koningii*	
31TA, 32sub	O	*T. koningiopsis*	
74, 95	Ko, H	*T. spirale*	
38Asub	O	*T. tomentosum*	
41	Ko	*Umbelopsis changbaiensis*	Described as potential biocontrol agent
56	Wp	*U. ramanniana*	
91	R	*U. vinace*	
15sub	O	*Verticillium* sp.	

a Ka = Ohakune, Manawatu-Whanganui; Ko = Kohatu, Nelson; P = Whangarei, Northland; KR = Kikiwa, Nelson; W = Inland Tokomaru Bay, Gisborne; M = Banks Peninsula, Canterbury; Wp = Inland Whatatutu, Gisborne; O = Kaikohe, Northland; R = Rakaia, Canterbury; T = Kawerau, Bay of Plenty.

b Species identification based on analyses of *tef1* and ITS sequences for *Trichoderma* and other fungi genera, respectively.

Table 3: Fungi identification of greenhouse/nursery *P. radiata* root isolates

Isolate Code	Collection Locality Code [a]	Identification [b]
P8, H12	GB	*Alternaria alternata*
H1	GB	*Aspergillus fumigatus*
101	GA	*As. ochraceus*
P1, P5	GB	*Botrytis cinerea*
H2	GB	*Chaetomium brasiliense*
H8	GB	*Cladosporium sphaerospermum*
H8	GB	*Epicoccum nigrum*
P1	GB	*Fusarium* sp.
P2, P10, P15, P16, H2, H5, H7 and H13, 90	GB, GA	*F. oxysporum*
P1	GB	*F. moniliforme*
H8	GB	*F. tricinctum*
P8	GB	*Geomyces pannorum*
P2, P8, P15, P18	GB	*Ilyonectria radicicola* or *macrodidyma*
P5, H2, H4	GB	*Paecilomyces* sp.
H12	GB	*Penicillium spinulosum*
P14	GB	*P. citreonigrum* or *toxicarium*
P1, P5, P10	GB	*Sclerotinia sclerotiorum*
H4	GB	*Sporothrix schencki*
P4, P19	GB	*Stagonosporosis cucurbitacearum*
108	GA	*Talaromyces verruculosus* or *pinophilus*
P4	GB	*Trichoderma asperellum*
P2, H19	GB	*T. atroviride*
P2, P14, H19	GB	*T. harzianum*

a GA = greenhouse/nursery experiment, Lincoln University (Whelan and Hill, 2017), GB = greenhouse/nursery experiment, Lincoln University (Brookes, 2014).

b Species identification based on analyses of primers ITS4/ITS5 and Ab28/Tw81/ITS1/ITS4 sequences for samples in the Whelan and Hill (2017) and Brookes (2014) studies, respectively.

Many endophytic species are recognised as weak or latent plant pathogens and may cause disease under certain conditions (Hyde and Soytong, 2008). This study provides additional information on the natural endophytes in New Zealand plantation systems that may become potentially emergent fungal pathogens. This is particularly important with the current changes to environmental conditions. Global climate change may lead to warmer growing temperatures in New Zealand plantations and induce warm-tolerant latent endophytes to become pathogenetic (e.g. *Erythricium salmonicolor,* a species of the family *Corticiaceae,* that causes wood cankers in tropical citrus and rubber crops). *Resinicium bicolor, a* pathogen of Douglas fir (*Pseudotsuga menziesii*) may need to be monitored for any change in its impact in *P. radiata* plantations.

Studies of the root microbiome require efficient methods that maximise the recovery of culturable endophytic fungi from surface-sterilised roots. The culturing method of tissue culture plates filled with agar is novel compared to the traditional use of petri dishes and was developed to improve the:

- convenience of plate preparation and handling;
- efficiency of agar use (1 mL per well for tissue culture plate versus 30 mL for petri dish); and
- separation of fungal colonies by confining fast-growing colonies to individual wells.

This tissue culture method is recommended for microbiome studies involving large-scale sampling of plant tissues.

The ethanol/sodium hypochlorite washing method used in this study provided adequate sterilisation of root surfaces with only 3% of control plates (2 of 60 plates) producing a colony growth. Therefore, the majority of fungal species identified are likely to have been within the roots. The direct-culturing approach, however, to accurately confirm presence of fungal species, can be problematic. Aspects of the isolation methods, including culturing temperature and type of isolation media, are likely to select for faster-growing or otherwise more competitive species (e.g. *Mucor, Rhizopus, Neurospora* sp.) which may obscure the recovery of slower-growing or less competitive species. These isolation conditions may indicate fungal microbes that flourish in warm, nutrient-rich environments but may not represent the microbes that can grow in more extreme conditions.

CONCLUSIONS AND RECOMMENDATIONS

This study provides new information for the establishment of the baseline endophytic root fungal communities present in *Pinus radiata* plantation trees in New Zealand. Knowledge of endophyte composition and diversity in *P. radiata* will contribute to enhanced understanding of the role of these fungi. Sequence-based identifications revealed a diverse population of fungal species in natural association with plantation *P. radiata* trees, including at least 57 species. Species diversity was increased when data for the two greenhouse studies were included, with 69 fungal species being identified. In addition to providing significant information on biodiversity, this study has led to a well-characterised culture collection available for future fungal interaction research and biocontrol screening.

Further isolation of endophytic fungi from additional trees, as well for other New Zealand forest areas, is likely to reveal more species of interest. Further work is required to determine the impact and function of the endophytes identified in this study on specific relationships with *Trichoderma* strains used in biocontrol of fungal disease in New Zealand. Once these relationships are better understood, there is potential for modification of the endophyte population to influence both the micro-organisms that either benefit or hinder the applied *Trichoderma* strains and those micro-organisms that contribute to the survival of disease pathogens.

ACKNOWLEDGEMENTS

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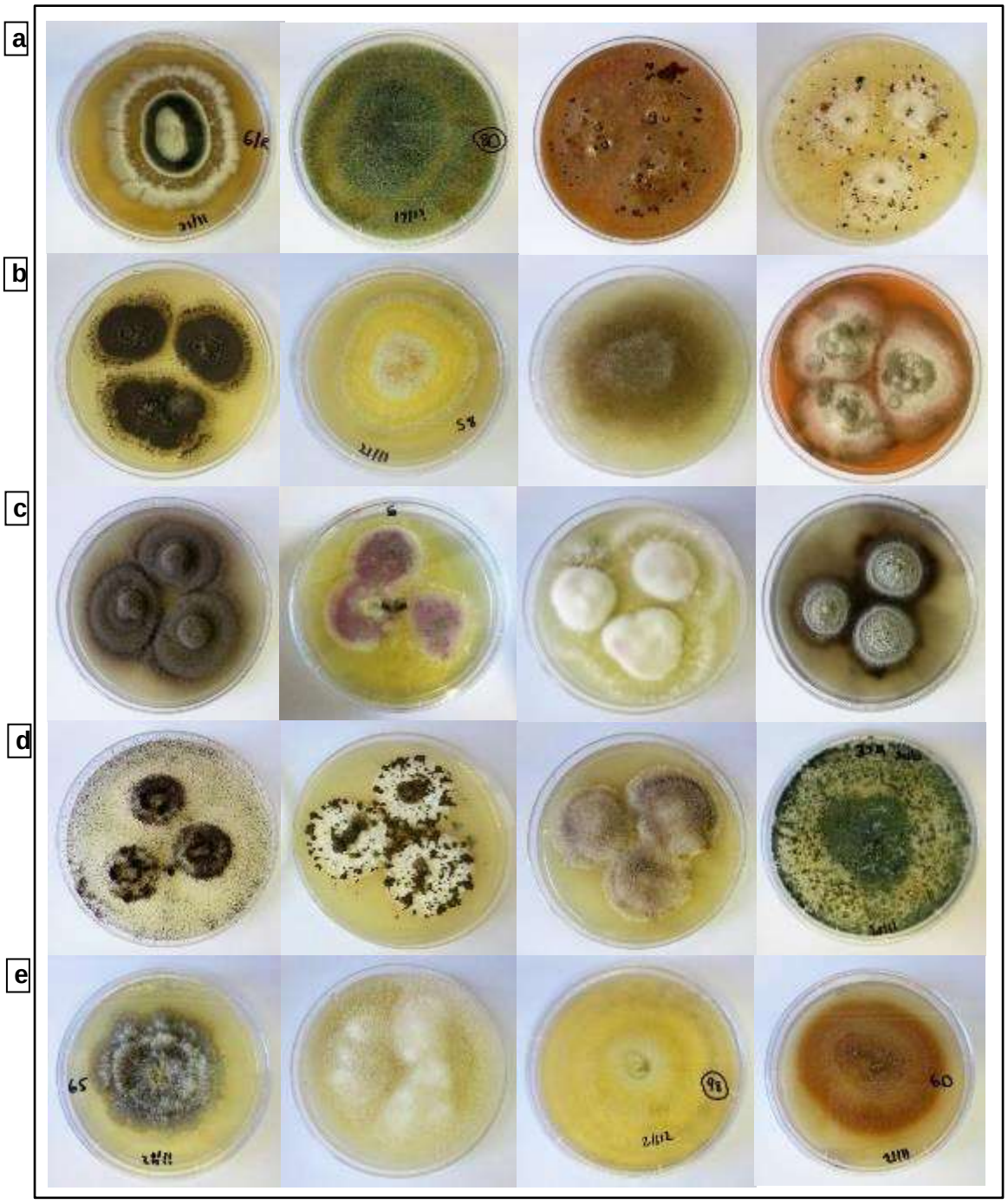
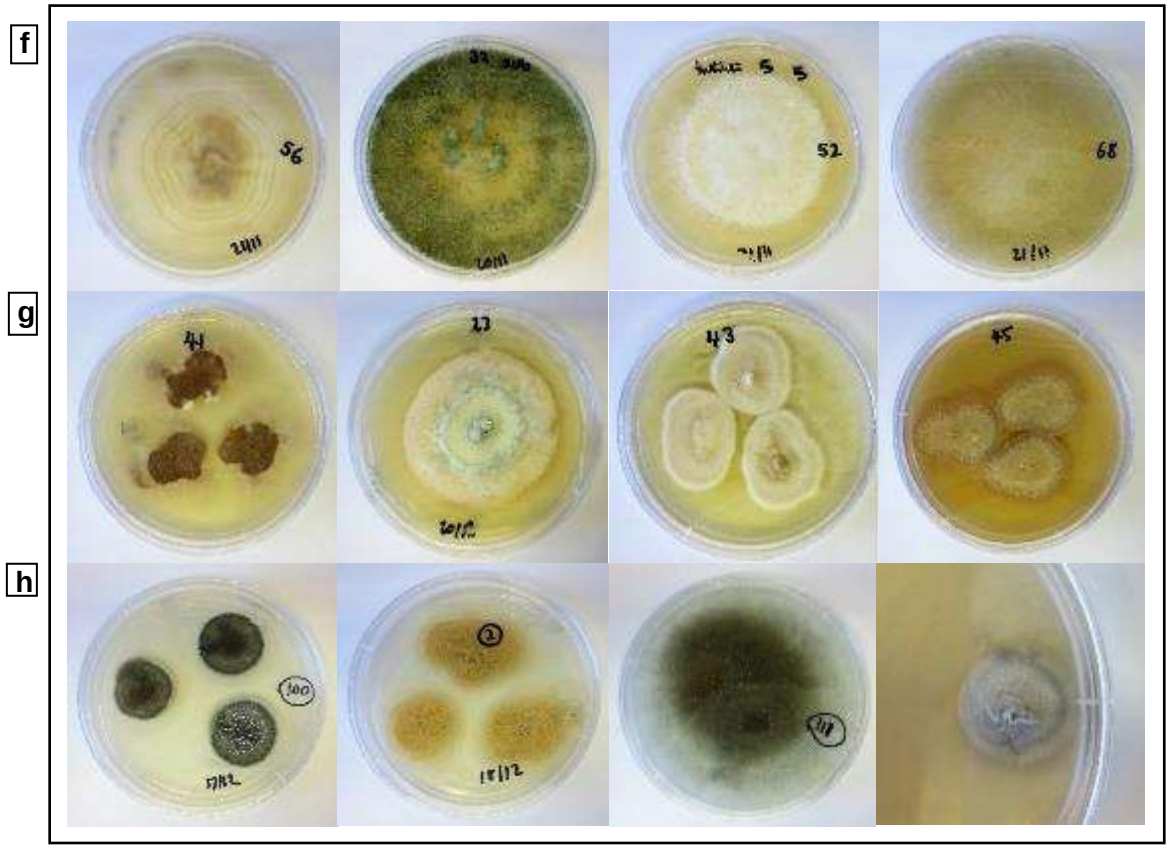
APPENDIX



Appendix A: Fungal isolates sub-cultured from surface-sterilised *P. radiata* roots plated on MYE agar. Row a (left to right): *Fusarium lateritium* or *avenaceum*, *Trichoderma crassum, Ilyonectria radicicola* and *Pestalotiopsis disseminata* ; Row b: *Talaromyes acaricola*, *Epicoccum nigum, Absidia psychrophilia* and *Penicillium sanguifluum*; Row c: *Cladophialophora chaetospira, F. oxysporum*, *Verticillium* sp. and *Hyaloscyphaceae* sp; Row d: *Aspergillus niger* and *As. welwitschiae, P. amaliae, Cunninghamella elegans* and *Trichoderma hamatum* ; Row e: *Phacidiopyenis washingtoneis*, *Gongronella butleri, Mucor moelleri* and *I. cyclaminicola*.



Appendix A continued: Fungal isolates sub-cultured from surface-sterilised *P. radiata* roots plated on MYE agar. Row f (left to right): *Umbelopsis ramanniana*, *T. koningiopsis, F. avenareum* and *M. hiemalis*; Row g: *U. changbalensis, P. canescens, Mortierella* sp. and *As. cerninus;* Row h: *Phialocephala fortinii, As. ochraceus, Ab. Glauca and Rhizoscyphus* sp.