Screening of *Agathis australis* endophytes as biological control agents against kauri dieback pathogen *Phytophthora agathidicida*

Praveenth Lawrence, Lauren Jackson, Mahajabeen Padamsee, Kevin Lee & Donnabella Lacap-Bugler

Abstract

Agathis australis (Kauri) is a significant and iconic native tree of Aotearoa, New Zealand. A disease known as kauri-dieback is currently affecting kauri trees. The aetiology of the disease has been associated with *Phytophthora agathidicida* (PA) which poses severe threat to kauri at individual and population levels. Currently, the only treatment available is the injection of phosphite into the trees. We investigated the potential of fungal endophytes to influence the growth of PA. Seventeen previously isolated endophytic fungi from kauri roots were examined morphologically and the antagonistic effects against the plant pathogen. Five of the 17 fungal endophytes tested demonstrated growth suppression of the pathogen in dual culture. *Pezicula* sp. partly inhibited the growth of *P. agathidicida* whilst *Coprinellus micaceus* and *Ilyonectria mors-panacis* completely inhibited the growth of the pathogen. Oospores were not observed in the dual culture nor was the *P. agathidicida* viable when transferred into fresh culture media. This study illustrates that naturally occurring fungal species in kauri roots can suppress the growth of PA. This finding offers a possibility of a biological control for dieback disease in kauri which may contribute to the mitigation of natural disease management and biocontrol of plant diseases without compromising the natural ecosystem.

Introduction

Agathis australis (kauri) is endemic to Aotearoa, New Zealand and belongs to the ancient family of coniferous trees, Araucariaceae. Māori have a significant relationship with kauri as it is considered as one of their taonga (treasure) which is also evident in their Māori traditions, artwork, folktales, and legends. Virgin kauri forests then covered more than 1.5 million hectares of Aotearoa/New Zealand (Halkett 1983). Subsequently, due to the its timber appearance and working properties the kauri timber and gum industries were established (Cheeseman et al. 2011; Steward 2011). This led to the harmful misuse of the species, completely changing the upper North Island's forest landscape (Steward 2011). Large proportion of the kauri forest was also transformed into farmlands. To date, only 7,500 hectares of primary virgin kauri forests remains, and 60,000 hectares of secondary kauri forests, or savannah containing regenerating kauri. Much of these forests are restricted to remote locations on the Great Barrier Island, Coromandel Peninsula, and the North Auckland Peninsula (Halkett 1983).

At present, a *Phytophthora* disease, known as kauri dieback, is threatening the existence of kauri. *Phytophthora agathidicida* is the primary causal agent of the kauri-dieback disease throughout lowland stands in Northern New Zealand (Beever et al. 2007; Weir et al. 2015). *P. agathidicida* infects the roots and damages the tissues that distribute nutrients within the tree to cause collar rot in all age groups of kauri trees (Beever et al. 2007). Several symptoms arise as a result, including the characteristic root and collar rot, resin exuding lesions, severe chlorosis, canopy thinning and widespread tree mortality (Waipara et al. 2013; Bassett et al. 2017). Considering the current distribution of kauri forests and the widespread dispersal of *P. agathidicida* throughout major kauri stands (e.g., Waitakere Ranges and Waipoua Forest) in Northern New Zealand, this pathogen poses a significant threat to the long-term survival of this iconic tree species (Waipara et al. 2013; Bassett et al. 2017).

Control agents such as phosphite effectively control many plant diseases caused by *Phytophthora spp.* (Horner and Hough 2013, 2014). Likewise, in recent glasshouse studies on 2-year-old kauri seedlings, phosphite protected *P. agathidicida*; phosphite injection into the trunk resulted in a 100% survival rate following soil inoculation. Despite the evidence for phosphite protection against *P. agathidicida*, there are ongoing concerns with phytotoxicity and interrogations about the commercial practicability of widespread use of phosphite as a control measure. Phytotoxic symptoms (e.g., leaf yellowing) were observed in 20% of phosphite-injected individuals (Horner and Hough 2014). Furthermore, applying phosphite to entire kauri forests was recognized as unsustainable given the huge costs that would be involved (Horner and Hough 2014). Therefore, fungicide usage (*i.e.*, phosphite) will likely only supplement other disease management strategies currently in place to mitigate kauri dieback, with a specific focus on shielding the largest and most notable kauri individuals.

One approach gaining more attention is the role of endophytes in plant health. Endophytes are microorganisms that live inside plant tissues and are not harmful to the host plant (Hallmann et al. 1997). Endophytes inhabit most plant and have been isolated from various plants species (Ziadi et al. 2016). They live in intra- and intercellular spaces of the plant tissue interacting with the hosts, and a diverse array of species has been reported to be endophytic (Elbeltagy et al. 2001). Colonization may take place at the local tissue level or throughout the plant, with microbial colonies and biofilms residing latently in the intercellular spaces and inside the vascular tissues (Gage et al. 1996; Hinton and Bacon 1995; Gopalaswamy et al. 2000; Sessitsch et al. 2002). Sturz and Nowak (2000), proposed that these endophytes originated from the rhizosphere or phylloplane micro-flora and observed that many rhizosphere bacteria could penetrate and colonize root tissue, providing a route into the xylem. In this vascular tissue, the microbes could transport themselves throughout the plant and colonize it systemically. Once inside the plant, endophytic populations have been

observed to grow between 2.0 and 7.0 log10 cells per gram of fresh tissue (Shishido et al. 1999; McInroy and Kloepper 2007).

Specific endophytes have demonstrated to enhance plant health. The fungal endophyte from Yew tree *Paraconiothrium* SSM001 increase plant resistance to pathogens (Soliman et al. 2015). Norway spruce root endophyte *Phialocephala sphareoides* was able to inhibit phytopathogens *Heterobasidion parviporum*, *Phytophthora pini*, *Botrytis cinereal* under *in vitro* conditions and promote root shoot ratio (Terhonen et al. 2016). *Colletotrichum tofieldiae*, a fungal endophyte of *Arabidopsis thaliana*, transfers macronutrient-phosphorus to shoots and promotes plant growth (Shishido et al. 1999; Azevedo et al. 2000; James 2000; Sturz and Nowak 2000; Bacilio-Jiménez et al. 2001; Iwai et al. 2010; Hiruma et al. 2016).

Dark septate endophytes (DSE), belonging to the class 4 endophytes, colonize their hosts' roots (Rodriguez et al. 2009). They are conidial or sterile septate fungal endophytes that form melanized structures, including inter- and intracellular hyphae and microsclerotia in the roots (Jumpponen and Trappe 1998; Tellenbach et al. 2013). These assemblages were found in almost all natural ecosystems, particularly stressful environments. In some cases, several DSEs have been reported to exhibit tolerance to stressors under *in vitro* culture conditions (Likar and Regvar 2013; Zhan et al. 2015; Berthelot et al. 2016).

Biological control agents (BCAs), whose primary mode of action is through competition, sustaining high environmental population levels is essential to suppress target pathogens (Alabouvette et al. 2006). Moreover, the ecotoxicological risk and associated risk assessments required to facilitate their application are much lower as they inhibit pathogens through general ecological processes rather than the production of antimicrobial compounds (Köhl et al. 2019).

The diversity and the distribution of endophytes with the potential to be BCAs living in *A. australis* roots are poorly understood. In this study, we demonstrated formerly isolated fungal endophytes from *A. australis* roots to suppress the growth of *P. agathidicida*. This study is an initiative to screen endophytes for inhibiting the growth of *P. agathidicida* and highlights the need to further investigate their potential as biological control agents for the kauri dieback pathogen.

Materials and methods

Endophytes evaluated in this study were previously isolated from *A. australis* root tissues. (NSF Award Search: Award # 1613884—EAPSI: Identifying Fungi Associated with the Ancient Conifer Agathis Australis and Testing their Potential as Biological Control Agents against Harmful Pathogen). Root samples were taken from rickers (age range from 150 to 300 years) located at Waitakere ranges, and no tap roots were sampled in this study; fine roots were collected (approximately 3 g per sample). Roots

were washed, surface-sterilized, and cultured in Potato Dextrose Agar (PDA). All plates were incubated in ambient conditions at 20 ℃, and any growth was subcultured to obtain pure cultures for sequencing. The full ITS gene sequence data were submitted to GenBank with accession numbers listed in Table 1.

Table 1 Fungal endophyte isolates from *Agathis australis* roots and the closest BLASTn match

In vitro screening of fungal endophytes for bioactivity against *Phytophthora agathidicida*

Endophytic fungal isolates from kauri roots were cultured individually in clarified V8 juice Agar (cV8A). Media was prepared by adding 200 ml of Clarified V8 juice, 15 g Bacto agar (Difco) and 800 ml of distilled water and autoclaved at 121 °C for 15 min (Tuite 1969). cV8A was selected for this experiment due to its ability to induce the sporulation of *Phytophthora* species (Weir et al. 2015).

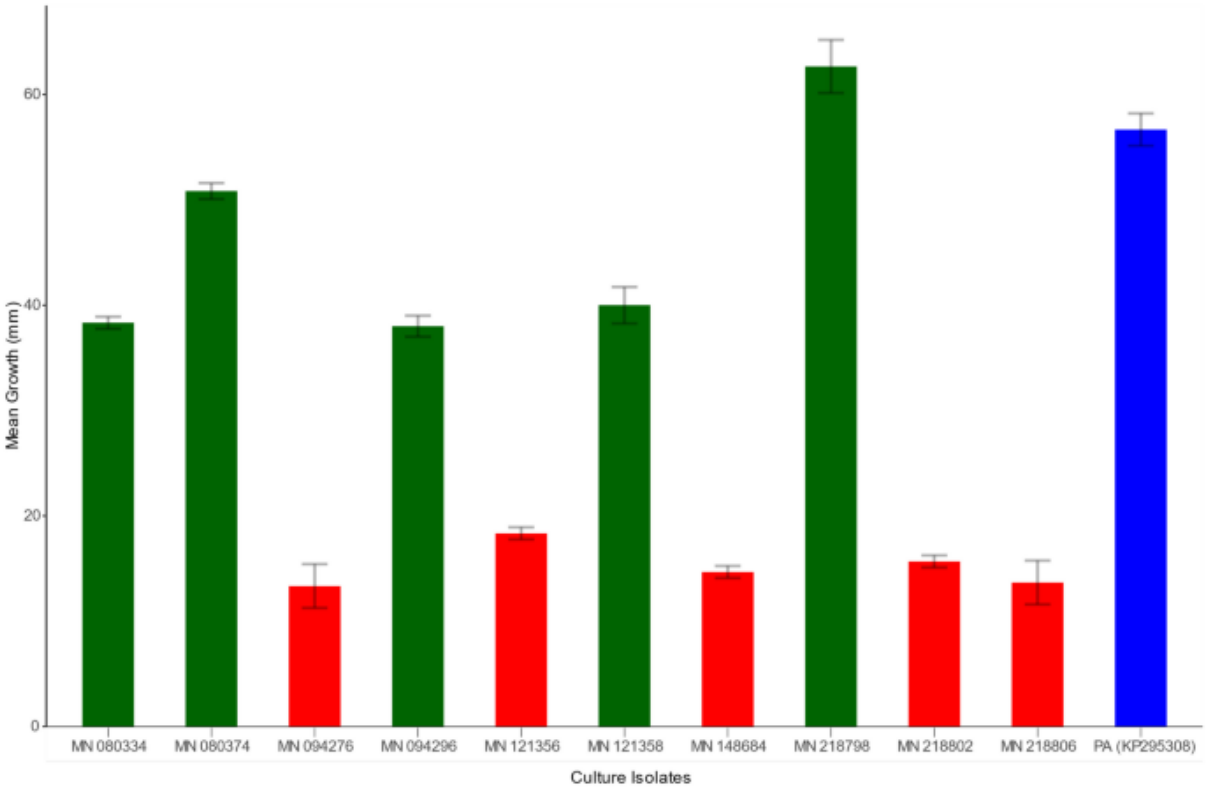
The 17 endophytic fungi (Table 1) and *P. agathidicida* were grown individually in cV8A, at 20 °C, in the dark in triplicates to obtain their growth rate. Radial growth (radius) of mycelia in millimetres was recorded every 24 h for 30 days. Endophyte cultures which were slow growing (less than 20 mm in 14 days) in cV8A were not considered in the dual-culture experiment. The growth rate of *Phytophthora agathidicida* was determined in cV8A culture media.

An *in vitro* dual culture method was employed in which a fungal endophyte isolate and PA were inoculated in the same Petri plate using agar plugs collected from eight to ten days old fungal cultures grown in cV8A. Endophyte isolate and PA were grown side by side in the same Petri plate using 5 mm diameter plugs. The *in vitro* screening was conducted in a growth chamber set at 20 ± 2 °C in triplicate. Control treatments had plugs of uncolonized media. The growth rate was measured every day for 35 days, and the endophyte growth inhibition effect on the pathogen were assessed using the formula:

$$\mathrm{Growth\;inhibition\;\%}=\frac{{\text{R}}2-{\text{R}}1}{{\text{R}}2}\times 100\%$$

in which R2 is PA mycelia radius in control plate (mm), R1 is PA mycelia radius in PA-endophyte dual culture (mm). Five endophytes (Fig. 1) were screened for potential as biological agents (BCAs) against kauri dieback pathogen *Phytophthora agathidicida*.

Fig. 1



Radial mean growth of endophyte isolates from *Agathis australis* roots and pathogen *Phytophthora agathidicida* in cV8A media at 20 °C in the dark on day 14. Red bars indicate the endophyte isolates which did not grew above 20 mm on day 14. Green bars indicate the endophytes isolates which grew above 20 mm on day 14. Blue bar is the growth of PA

Microscopic examination of the fungal structure was carried out using a compound light microscope (Leica DM750, image captured by Leica ICC50 HD camera and analyzed using Leica LAZ EZ software manufactured by Leica Microsystems (Schweiz) AG Max Schmidheiny-Str. 201 9435 Heerbrugg Switzerland). The viability and formation of oospore by the *P. agathidicida* in the dual culture was examined by taking an agar plug from the dual culture plate at the antagonistic zone where the endophyte (macroconidia) and pathogen (oospores) were present. The agar plug was inoculated on fresh cV8A plate and incubated at 20 °C, in the dark for 35 days to determine viability after dual culture with endophyte. Microscopic examination of the hyphae and fruiting bodies were carried out using compound light microscope.

Growth rate data analysis

The mean growth rate and percentage of growth inhibition among treatments and control were compared using the Student T-test at the 5% level (p = 0.05) of significance and presented as the mean values ± standard deviation (SD).

Phylogenetic analysis

The full ITS gene region was used in the analysis to determine the taxonomic identity and phylogenetic placement of the endophyte isolates. The National Centre for Biotechnology Information (NCBI) BLASTn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to infer the taxonomic identity of the isolates. Phylogenetic analyses were performed using the MEGA v11.0 software. The alignment was carried out using the Muscle algorithm of consensus ITS1, 5.8S rRNA gene, ITS 2 and large subunit of rRNA and was inspected in MEGA11 to ensure consistent reading frame, accurate gap placement, and reduction of sequence overhangs.

The model test module of MEGA 11 was used to ensure the accurate choice of substitution model and the evolutionary history was inferred using the maximum likelihood (ML) method (Harris and Stöcker 1998) based on a suitable substitution model (SYM + I + G4; corrected Akaike Information Criterion scores & weights) with 1000 bootstrap replicates. FigTree v1.4.4 was used to visualise and edit the ML tree.

The full ITS gene sequence data used in this study were deposited in the GenBank nucleotide database with the accession numbers provided in Table 1.

Results

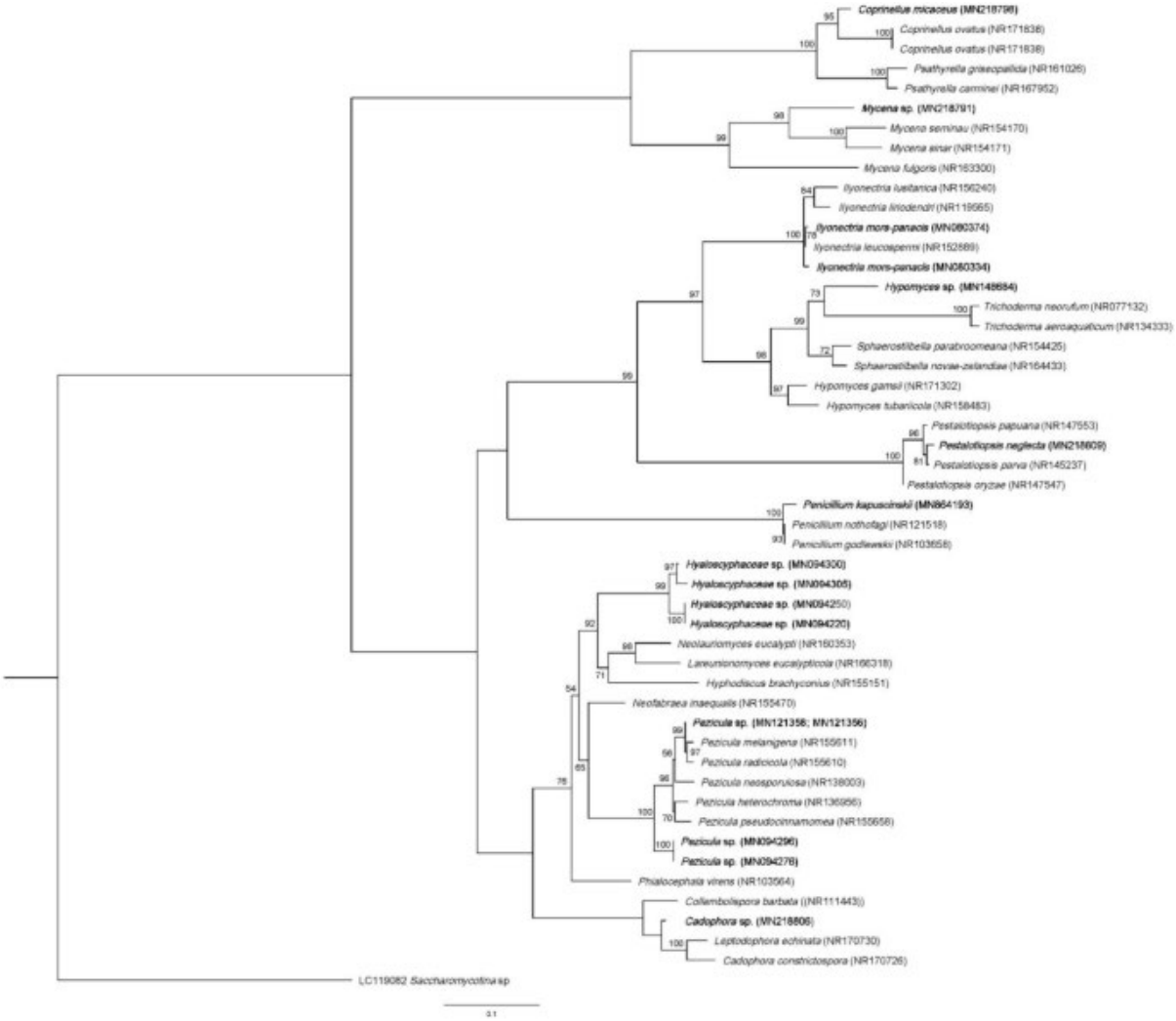
Seventeen endophyte isolates from the research, NSF Award Search: Award # 1613884—EAPSI: Identifying Fungi Associated with the Ancient Conifer *Agathis australis* and Testing their Potential as Biological Control Agents against Harmful Pathogen, were considered in this study (Table 1). Ten of the 17 grew on the cV8A media, and only five of these isolates were optimally grown (> 20 mm radial growth after 14 days at 20 °C in the dark) in the cV8A, which allowed the examination of their ability to inhibit the growth of the kauri dieback pathogen *Phytophthora agathidicida in vitro* (Fig. 1).

Clarified V8 juice agar was selected for the experiment as it induces sporulation and is favourable to *Phytophthora agathidicida*. Moreover, endophytes were selected based on the growth rate in cV8A media to study the endophyte behaviour in a favourable pathogen environment. Six slow growing endophyte isolates in cV8A did not show visible hyphae until day 14.

The taxonomic assignment of each fungal isolate was determined based on the closest full ITS gene region match using the NCBI BLASTn database (Table 1). The phylogenetic

analyses further support the delineation of these different isolate with > 50% bootstrap support (Fig. 2).

Fig. 2



Phylogenetic relationship among fungal ITS gene region. Fungal endophyte isolated from kauri roots are in bold. The tree topology is supported by bootstrap values for 1000 replications, shown for branches supported by more than 50% of the trees. The scale bars represent the nucleotide change per position

Isolates ICMP 21398 and ICMP 21347 could be the same species of *Pezicula* with 100% bootstrap support. Whilst ES107 and ICMP 21343 are identical isolates which are closely associated with the *P. melanigena* and *P. radicicola* with 99% support. ES91 and ICMP 21339 are grouped within the *Ilyonectria* sp. clade. ICMP 21358 is closely associated with *Pestalotiopsis parva* with 81% bootstrap support. ICMP 21377 has a closest BLAST match of *Sphaerostilbella novae-zealandiae* but also closely associated with *Trichoderma* sp. and *Hypomyces* sp. ICMP 21382 is associated with

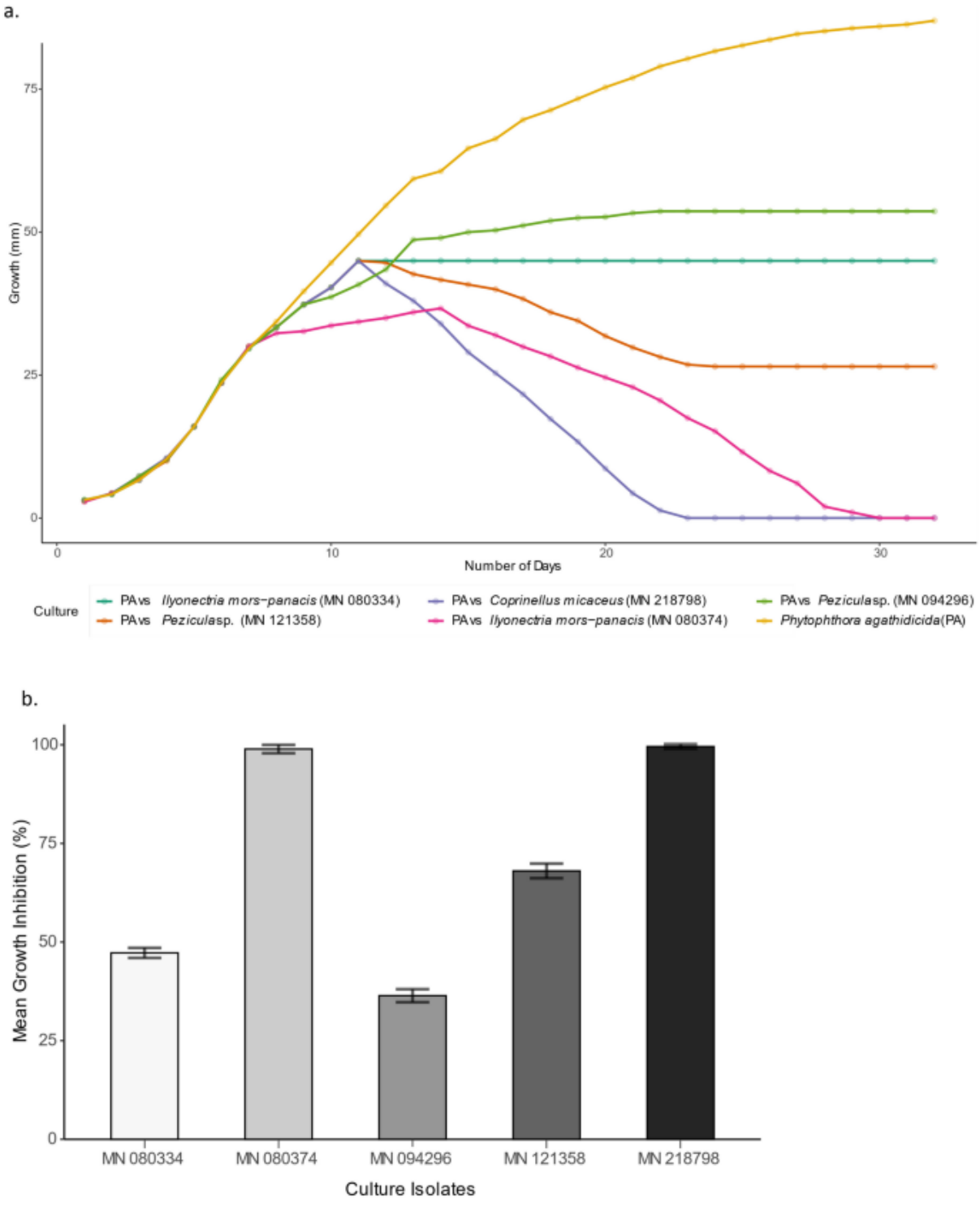
genus *Pencillium* sp., and ICMP 21392 is closely associated with *Leptodophora echinate* and *Cadophora constrictospora*. ICMP 21455 and LJ215 are within the Basidiomycota clade, particularly closely associate with *Coprinellus ovatus*, *Mycena seminau* and *Mysena sinar*, respectively. Lastly isolates ICMP 21345 and LJ 235; ICMP 21447 and LJ 308 are closely related with 100% and 97% bootstrap support, and all are associated with *Neolauriomyces eucalypti*, *Lareunionomyces eucalypticola* and *Hyphodiscus brachyconius*.

***In vitro* screening of endophyte fungi for bioactivity against pathogen PA**

All seventeen fungal endophyte isolates were initially grown in cV8A media. Ten endophytes were able to grow in cV8A media at 20 °C in the dark. The remaining seven fungal endophyte isolates did not grow in cV8A and were not considered in the dual-culture experiment. ICMP 21455 was the fastest growing (65 mm) endophyte in cV8A, and ICMP 21398 was the slowest (13 mm). ES107, ES91, ICMP 21339, and ICMP 21347 had mean growth of 41 mm, 38 mm, 51 mm, and 38 mm, and respectively, on day 14 (Fig. 1). Only the fungal endophyte isolates that grew > 20 mm on day 14 were selected for the dual culture experiment with pathogen PA (Fig. 1). *Phytophthora agathidicida* grew well more than 4.5 cm by day 14. The mean growth of PA in cV8A on day 14 incubation was 59 mm with a growth rate of 4.21 mm per day which gradually decreased from day 16 to 32 mm with an average growth rate of 1.33 mm per day.

Five fungal endophytes were employed for the *in vitro* dual culture with the pathogen *Phytophthora agathidicida* (Fig. 3). The five endophyte isolates exhibited various levels of inhibition against PA in dual cultures (Fig. 3b). *Coprinellus micaceus* (ICMP 21455) was most effective in suppressing PA growth with 100% growth coverage of the plate on day 24. *Ilyonectria mors-panacis* (ICMP 21339) suppressed the mycelial growth of PA and grew over the pathogen zone on day 31. *Pezicula* sp. (ES107) 6 CC-2015 inhibited the growth of the pathogen and occupied 69.30% of the plate. *Ilyonectria mors-panacis* (ES91) and *Pezicula* sp. (ICMP 21347) occupied 48.2% and 38.7% of the plate, respectively (Fig. 3b).

Fig. 3



Mean growth of *Phytophthora agathidicida* in the presence of endophyte isolate (**a**). Mycelial growth inhibition of *Phytophthora agathidicida* on day 32 in dual culture experiment (**b**)

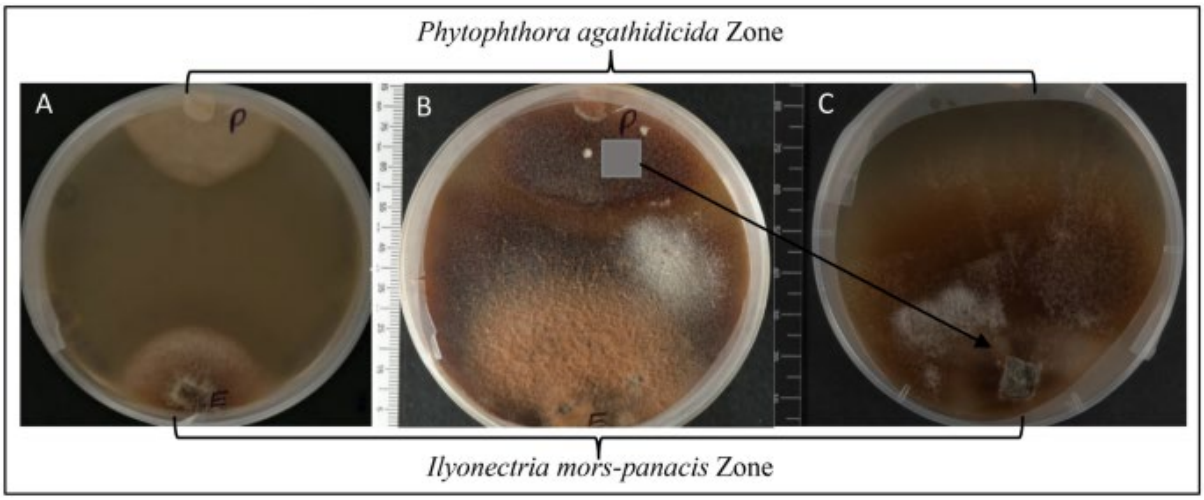
Furthermore, the growth of the pathogen was not altered for the first eight days in dual culture, although growth suppression became evident when the PA hyphae were in contact with the endophyte hyphae (Fig. 3a). C*oprinellus micaceus* (ICMP 21455), *Ilyonectria mors-panacis* (ICMP 21339) and *Pezicula* sp. (6 CC-2015) were found to penetrate the pathogen zone and suppressed its growth (SI).

Coprinellus micaceus (ICMP 21455) and *Ilyonectria mors-panacis* (ICMP 21339) covered the entire pathogen zone and suppressed the PA growth entirely by day 24 and 31 (SI). All isolates examined in dual cultures inhibited the growth of the pathogen significantly with a *p*-value < 0.001 using the two-sample student t-tests (Table 2).

Table 2 The mycelial inhibition values of *P. agathidicida* cultures in dual culture

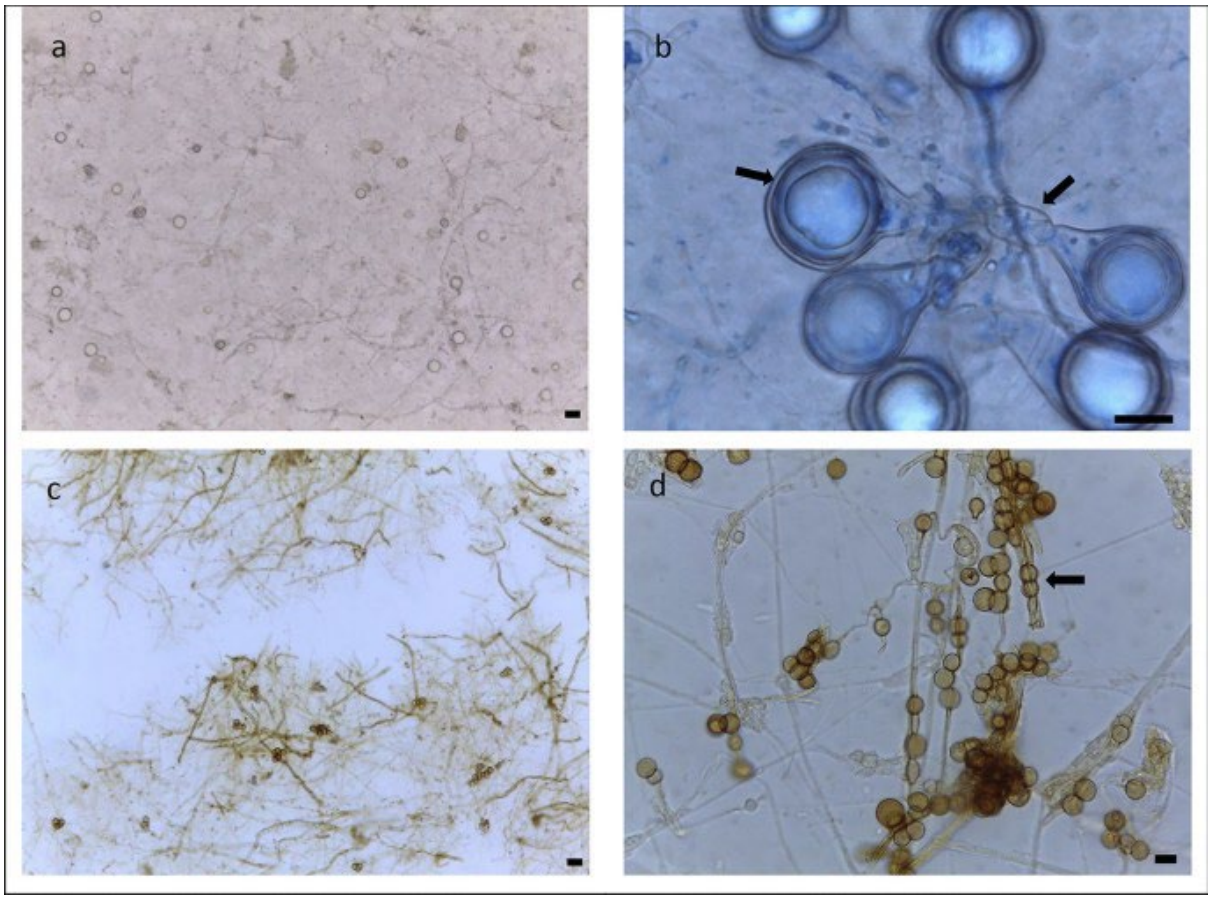
To determine the viability of the PA in the dual culture after the growth suppression of the endophyte isolate, an agar plug was obtained from the pathogen zone and cultured in fresh cV8A plate. Hyphae growth of endophyte was evident on day 2. The culture was allowed to saturate and incubated at optimum condition for 40 days or until the available nutrient has depleted. PA under nutrient depletion stress produce oospores. Microscopic examination of slides stained with bromophenol blue did not reveal observable oospores (Figs. 4 and 5).

Fig. 4



Viability and induce oospore formation test. *P. agathidicida* and endophyte *Ilyonectria mors-panacis* in dual-culture experiment (**A**). Endophyte over growing on *P. agathidicida* (**B**), where agar plug was isolated and inoculated in fresh cV8A culture media incubated for 40 days until the culture plate was saturated with hyphae (**C**)

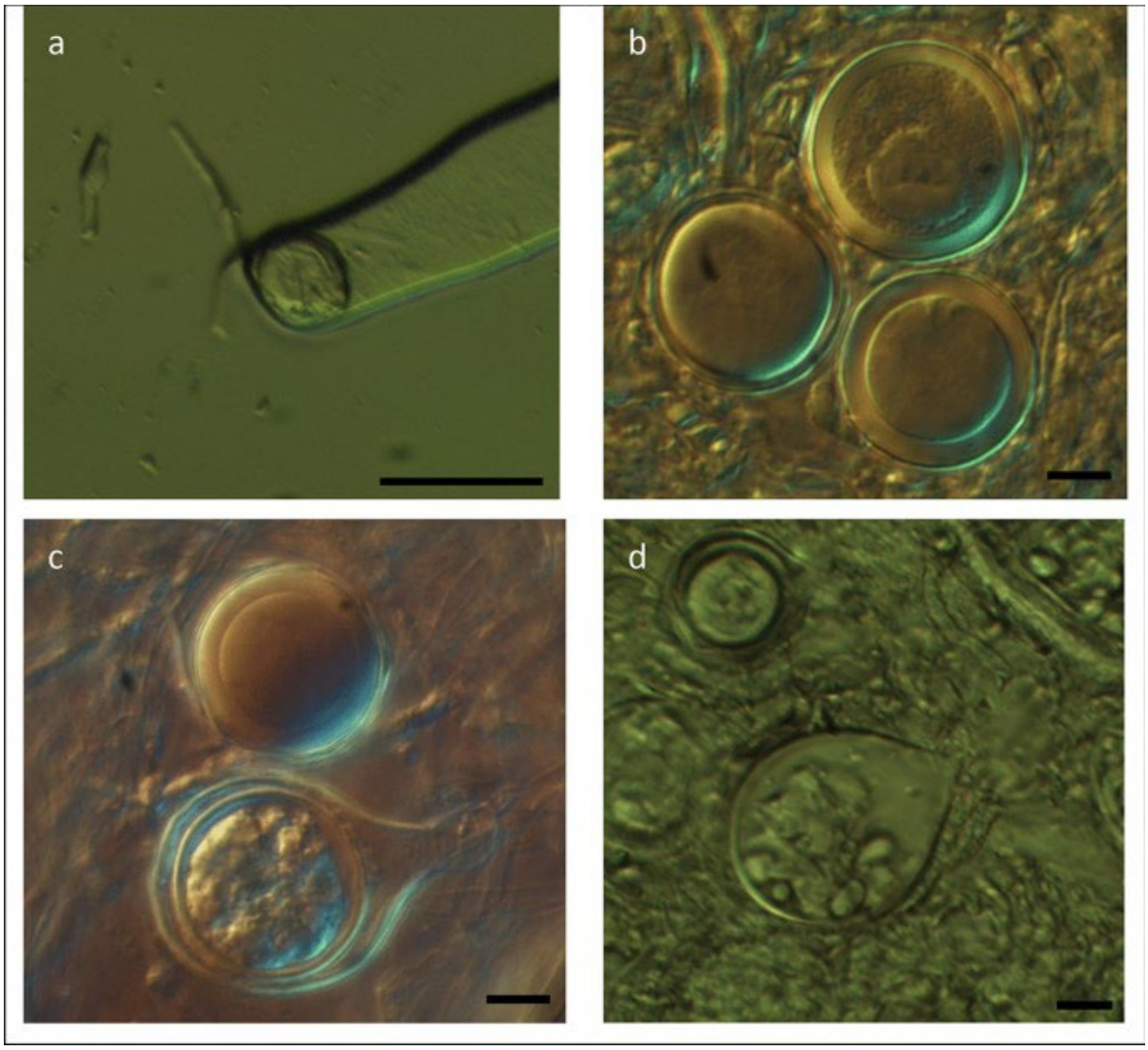
Fig. 5



Phytophthora agathidicida mycelia grown in pure culture at 100 × magnification (**a**), oogonia with amphigynous, sub-globose antheridia stained with bromophenol blue at 1000 × magnification (**b**). Dual culture of *P. agathidicida* and *Ilyonectria mors-pancis* mycelia at 100 × magnification (**c**), specimen stained with bromophenol blue with no evidence of *P. agathidicida* oogonia only chlamydospore of *Ilyonectria* at 400 × magnification (**d**). Scale bar is 10 µm

PA oogonium ornamentation is irregular and slightly raised projections larger than the oospore diameter. PA is homothallic, with *in vitro* isolates forming oogonia rapidly (3—4 days) and abundantly on cV8A in 3—4 days (Weir et al. 2015). Oogonia are globose with a width ranging between (22—45) µm. Oogonium wall ornamentation is mildly stipulate. Oospores nearly fill the oogonia with a width ranging between 19—35 µm. Sporangia are globose to ovoid-ellipsoid, papillate, borne terminally from long thin branched. Sporangia are non-caducous. Sporangia have a width ranging between 12.4—50 µm, and PA lacks chlamydospores in culture (Fig. 6) (Weir et al. 2015).

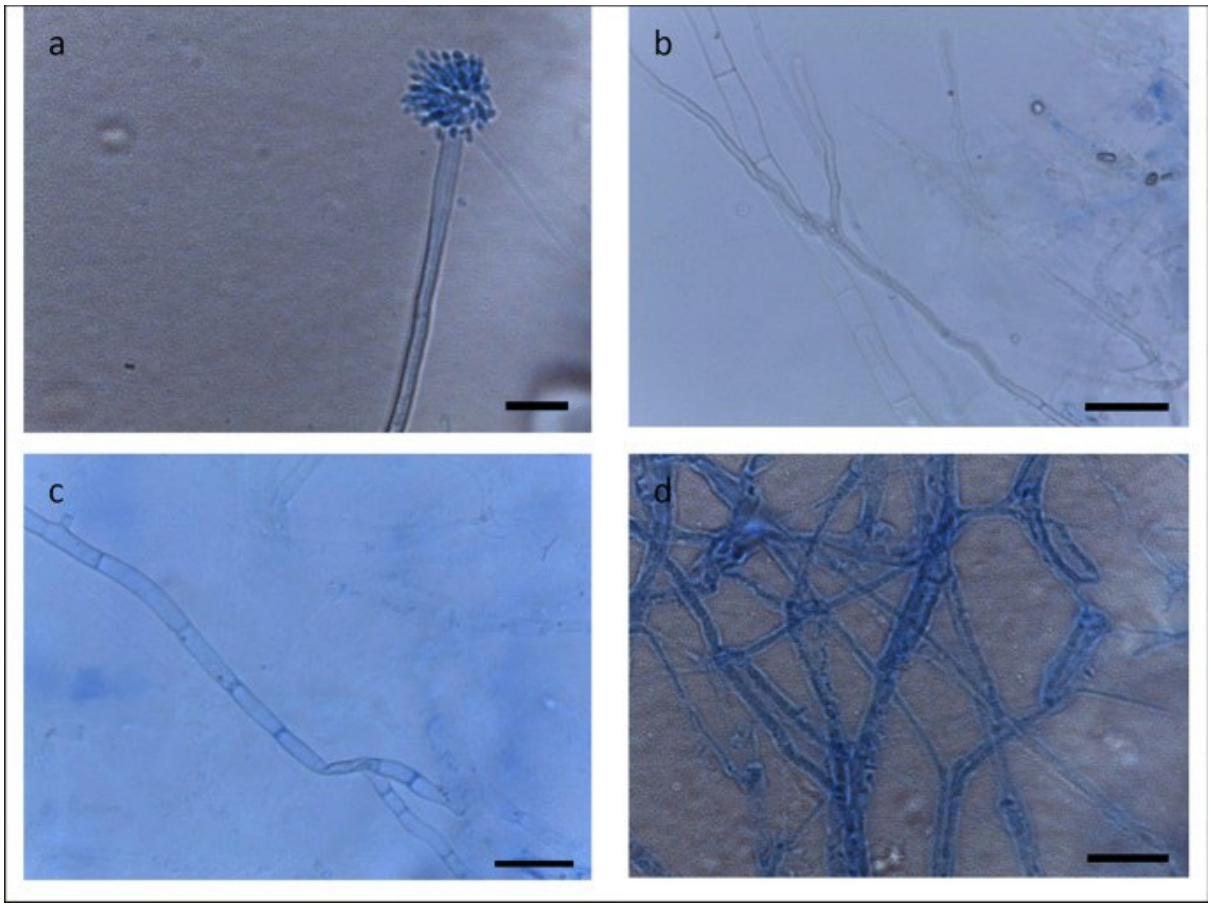
Fig. 6



Life cycle stages of *Phytophthora agathidicida*. Biflagellate zoospore (**a**), oospores (**b**) sporangia (**c**) and oogonia (**d**). Scale bar is 10 µm

Coprinellus micaceus was microscopically examined from the dual culture. The culture had high density of mycelia covering the entire plate, and air hyphae were also observed. Clamp cells and some septa were also observed but overall occurred at very sparse (Fig. 7).

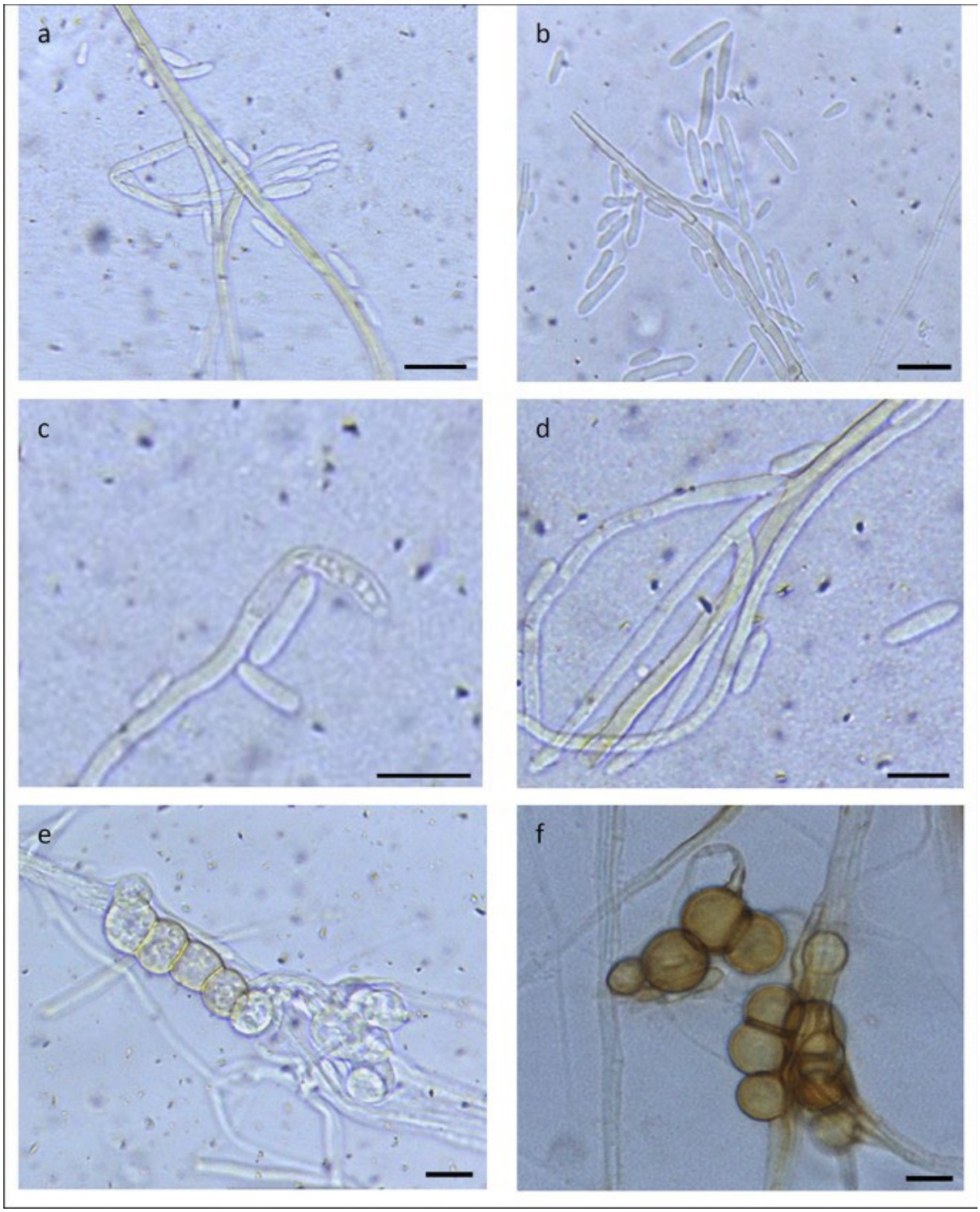
Fig. 7



Coprinellus micaceus odiophores (**a**), hyphae (**b**, **c**, **d**) stained with bromophenol blue. Scale bar is 10 µm

Furthermore, microscopic examination of *Ilyonectria mors-panacis* isolate on dual culture showed simple sporodochial conidiophores which were loosely aggregated, unbranched or lightly branched, with one to three septa, and cylindrical to subulate. Some complex conidiophores accumulated in small sporodochia and occasionally branched. Macroconidia were predominant with one to three septa, straight, cylindrical with nearly rounded ends and mainly without a hilum. Microconidia were almost straight and spheroidal to subcylindrical (Fig. 8). The chlamydospores observed were spherical or subspherical and 8–16 µm in diameter. Chlamydospores are thick-walled, found in chains or clumps and medium brown in colour (Fig. 8e, f).

Fig. 8



Ilyonectria mors-panacis simple conidiophores with aerial mycelium (**a**), micro and macro conidia (**a**, **b**, **c**, **d**), chlamydophores on mycelium (**e** and **f**). Scale bar is 10 µm

Discussion

Most plant species studied up to date have been found to host endophytes. Interactions between plants and endophytes may be symbiotic, mutualistic, and other types of relationships without causing symptoms or causing harm to their host. Endophytes colonize the same environment as plant pathogens and share an intimate relationship with the host plants. Thus, endophytes are a valuable natural resource for potential utilization as biological control agents (Stone et al. 2000; Tan and Zou 2001; Schulz et al. 2002; Zilber-Rosenberg and Rosenberg 2008). In our *in vitro* dual culture assay, root endophytes of *Agathis australis* exhibited evident growth suppression of kauri dieback pathogen *Phytophthora agathidicida*.

The ten endophytes that had significant growth in cV8A with radial mycelial growth of > 20 mm on day 14 were considered for the dual culture. These endophytes had relatively similar growth rate as PA. According to Lopez-Llorca and Duncan (1988), nutrient content is significant in the expression of fungal reproductive structures in culture media. A high concentration of carbohydrates presents in the medium or the absence of some minerals may contribute to the development of specific fungal species. The presence of specific ions and nutrient depletion in axenic culture led to the production of sporangia by *Phytophthora cinnamomi*. Nevertheless, it is not known whether the poor performance of other endophyte isolates could be attributed to the high concentration of carbohydrates or the lack of some minerals in the cV8A culture media. However, the role of nutrients in the fungal growth *in vivo* could be investigated.

Four isolates from Ascomycota and one isolate from Basidiomycota were able to grow > 20 mm on day 14. The Ascomycota isolates are *Ilyonectria mors-panacis* and *Pezicula* sp. *Ilyonectria* species generally had a reasonable growth rate in cV8A (Goh et al. 2022).

One Basidiomycota isolate, *Coprinellus micaceus* has ecological significance because it can live in poor nutrient ecosystems and create interactions with bacteria to overcome nutrient stress and feasible environmental conditions (Velez et al. 2018; Marini-Macouzet et al. 2020). *Coprinus* species are known for degrading monomeric phenolic and chloroaromatic compounds (Guiraud et al. 1999).
Moreover, *Coprinus* species were reported to exhibit high tolerance to high nitrite concentration, ammonium and urea, and were only inhibited at high $NaNO_2$ concentrations (Hintikka and Niemi 1999).

Two isolates, *Coprinellus micaceus* (ICMP 21455) which was fast growing compared to PA (72 mm in 14 days), and *Ilyonectria mors-panacis* (ICMP 21339) overgrew the mycelium of *P. agathidicida* in dual culture. These isolates were most effective in suppressing *P. agathidicida* growth with 100% growth coverage of the plate on day 24 and day 31, respectively, suggesting that these isolates have a faster growth rate and

were not inhibited by the presence of *P. agathidicida*. Furthermore, these endophytes outcompeted the PA on the cV8A dual culture. Micaceol, a sterol and (*Z*,*Z*)-4-oxo-2,5-hetpadienedioic acid were extracted from *Coprinus micaceus* from the Canadian Prairie region that demonstrated antimicrobial activity against pathogens *Corynebacterium xerosis* and *Staphylococcus aureus* and exhibited glutathione S-transferase inhibition (Zahid et al. 2006). Furthermore, gas chromatography and mass spectrometry studies of *C. micaceus* extracts in ethanol, chloroform and distilled water were reported to exhibit antimicrobial activities (Avcı et al. 2014). The culture filtrates of *Coprinus comatus* effectively reduced the disease severity of *Phytophthora* blight of pepper (Chen and Huang 2011). These studies suggests that *C. micaceus* isolated from kauri roots may produce chemical compounds that are pathogen inhibitors.

Coprinus micaceus and *I. mors-panacis* outcompeted the pathogen in cV8A dual culture set up which could present competition for space and nutrition. Further microscopic examination of the pathogen region in the dual culture plate showed no evidence of *P. agathidicida* oospores in the presence of *Ilyonectria mors-panacis*. This indicates that *I. mors-panacis* inhibits the regeneration of PA oospores. In an Australian shrub study, *Ilyonectria* species also exhibited antagonistic qualities against *Phytophthora cinnamomi* in *in vitro* dual culture (Andres et al. 2022).

However, *in vitro* results do not always translate the disease suppression in plants. Studies suggested that *in vitro* assays can be used as a rapid technique for selecting promising biological control agents (BCA) (Rajkumar et al. 2005). According to Pal and Gardener (2006) and Latha et al. (2009), the most effective BCA inhibit plant pathogens by multiple mechanisms of action, including the production of antimicrobial compounds, competition for space and nutrients, plant growth promotion and induced systemic resistance. Byers et al. (2021) identified *Penicillium* and *Burkholderia* soil isolates demonstrating inhibition towards *Phytophthora agathidicida* and suggesting these species were promising BCA. Our dual culture results suggest that *Coprinellus micaceus* and *Ilyonectria mors-panacis* may have multiple modes of action in pathogen suppression, such as competition for space or substrate or nutrients or production of metabolites antagonistic to *Phytophthora* or production of antimicrobial substance. Further studies are needed on the endophyte's mechanism of action is supressing pathogens. Future research should measure the impact of endophytes on *P. agathidicida* on sporangia production and zoospore release to quantify their inhibitory potential better and specific research is required to characterize the metabolites with potential antimicrobial action, as it is essential to fully understand their mode of action before they can be applied as BCA (Spadaro and Gullino 2005).

Conclusion

Kauri roots harbour endophytic fungi that can suppress growth and reproductive metabolism of plant pathogen such as *P. agathidicida*. In this study we identified and successfully cultured ten isolates in cV8A media that favours the growth of the plant pathogen. Five out of the ten isolates demonstrated different level of PA growth inhibition in the dual culture experiment. Of the 17 endophytes we identified four Ascomycota and one Basidiomycota endophytic isolates that can significantly inhibit the growth of *Phytophthora agathidicida* in *in vitro* dual cultures. *Coprinellus micaceus* and *Ilyonectria mors-panacis* not only inhibited the growth of PA it also suppressed the zoospore formation of *P. agathidicida*. Further studies need to be conducted on the mechanism of inhibition and possible metabolite production of these isolates as they can be considered potential biocontrol agent for plant pathogen such as *P. agathidicida* in kauri.

Data availability

Submission of sequence data into NCBI is publicly available.

References

Alabouvette C, Olivain C, Steinberg C (2006) Biological control of plant diseases: The European situation. Eur J Plant Pathol. https://doi.org/10.1007/s10658-005-0233-0

Andres SE, Emery NJ, Rymer PD, Powell JR (2022) Soil chemistry and fungal communities are associated with dieback in an Endangered Australian shrub. Plant Soil. https://doi.org/10.1007/s11104-022-05724-7

Avcı E, Alp Avcı G, Ali Köse D (2014) Determination of antioxidant and antimicrobial activities of medically important mushrooms using different solvents and chemical composition via GC/MS analyses. J Food Nutri Res. https://doi.org/10.12691/jfnr-2-8-1

Azevedo JL, Maccheroni W, Pereira JO, De Araújo WL (2000) Endophytic microorganisms: A review on insect control and recent advances on tropical plants. Electron J Biotechnol. https://doi.org/10.2225/vol3-issue1-fulltext-4

Bacilio-Jiménez M, Aguilar-Flores S, Del Valle MV, Pérez A, Zepeda A, Zenteno E (2001) Endophytic bacteria in rice seeds inhibit early colonization of roots by Azospirillum brasilense. Soil Bio Biochem. https://doi.org/10.1016/S0038-0717(00)00126-7

Bassett IE, Horner IJ, Hough EG, Wolber FM, Egeter B, Stanley MC, Krull CR (2017) Ingestion of infected roots by feral pigs provides a minor vector pathway for kauri dieback disease Phytophthora agathidicida.
Forestry. https://doi.org/10.1093/forestry/cpx019

Beever RE, Waipara NW, Ramsfield TD, Dick MA, Horner IJ (2007) Kauri (*Agathis australis*) under threat from *Phytophthora*? Phytophthoras in forests and natural

ecosystems. Proceedings of the Fourth Meeting of the International Union of Forest Research Organizations (IUFRO), Monterey, California, pp 74–85

Berthelot C, Leyval C, Foulon J, Chalot M, Blaudez D (2016) Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. FEMS Microbiol Econ. https://doi.org/10.1093/femsec/fiw144

Byers AK, Condron L, O'Callaghan M, Waipara N, Black A (2021) Identification of Burkholderia and Penicillium isolates from kauri (Agathis australis) soils that inhibit the mycelial growth of Phytophthora agathidicida. N Z Plant Prot. https://doi.org/10.30843/nzpp.2021.74.11736

Cheeseman TF, Hemsley WB, Smith M (2011) Illustrations of the New Zealand flora. Wellington, John Mackay, Govt. Printer, 1914. https://doi.org/10.5962/bhl.title.12029

Chen J, Huang J (2011) Antimicrobial activity of edible mushroom culture filtrates on plant pathogens. Plant Pathol Bull 19(4):261–270

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, Isawa T, Mitsui H, Minamisawa K (2001) Endophytic colonization and in planta nitrogen fixation by a herbaspirillum sp. isolated from wild rice species. App Environ Microbiol. https://doi.org/10.1128/aem.67.11.5285-5293.2001

Gage DJ, Bobo T, Long SR (1996) Use of green fluorescent protein to visualize the early events of symbiosis between Rhizobium meliloti and alfalfa (Medicago sativa). J Bacteriol. https://doi.org/10.1128/jb.178.24.7159-7166.1996

Goh J, Oh Y, Park YH, Mun HY, Park S, Cheon W (2022) Isolation and characterization of previously undescribed seventeen fungal species belonging to the order hypocreales in Korea. Kor J Mycol. https://doi.org/10.4489/KJM.20220001

Gopalaswamy G, Kannaiyan S, O'Callaghan KJ, Davey MR, Cocking EC (2000) The xylem of rice (Oryza sativa) is colonized by Azorhizobium caulinodans. Proc R Soc B: Biol Sci. https://doi.org/10.1098/rspb.2000.0973

Guiraud P, Steiman R, Ait-Laydi L, Seigle-Murandi F (1999) Degradation of phenolic and chloroaromatic compounds by coprinus spp. Chemosphere. https://doi.org/10.1016/S0045-6535(98)00479-2

Halkett J (1983) A basis for the management of New Zealand kauri (*Agathis australis* (D. Don) Lindl.) forest. N Z J For 28:15–23

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol. https://doi.org/10.1139/m97-131

Harris J, Stöcker H (1998) Handbook of mathematics and computational science. pp 823–824

Hintikka V, Niemi K (1999) Nitrite tolerance of different ectomycorrhizal and wood- and litter-decomposing fungi. Karstenia. https://doi.org/10.29203/ka.1999.337

Hinton DM, Bacon CW (1995) Enterobacter cloacae is an endophytic symbiont of corn. Mycopathologia. https://doi.org/10.1007/BF01103471

Hiruma K, Gerlach N, Sacristán S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramírez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root endophyte colletotrichum tofieldiae confers plant fitness benefits that are phosphate status dependent. Cell. https://doi.org/10.1016/j.cell.2016.02.028

Horner IJ, Hough EG (2013) Forest trials testing phosphate for the control of kauri dieback. Agrichem P Protection 12:7–12

Horner IJ, Hough EG (2014) Pathogenicity of four phytophthora species on kauri: *in vitro* and glasshouse trials. N Z Plant Prot 67:54–59

Iwai S, Chai B, Sul WJ, Cole JR, Hashsham SA, Tiedje JM (2010) Gene-targeted-metagenomics reveals extensive diversity of aromatic dioxygenase genes in the environment. ISME J. https://doi.org/10.1038/ismej.2009.104

James EK (2000) Nitrogen fixation in endophytic and associative symbiosis. Field Crop Res. https://doi.org/10.1016/S0378-4290(99)00087-8

Jumpponen A, Trappe JM (1998) Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. New Phytol. https://doi.org/10.1046/j.1469-8137.1998.00265.x

Köhl J, Kolnaar R, Ravensberg WJ (2019) Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00845

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009) Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani. Biol Control. https://doi.org/10.1016/J.BIOCONTROL.2009.03.002

Likar M, Regvar M (2013) Isolates of dark septate endophytes reduce metal uptake and improve physiology of Salix caprea L. Plant Soil. https://doi.org/10.1007/s11104-013-1656-6

Lopez-Llorca LV, Duncan GH (1988) A study of fungal endoparasitism of the cereal cyst nematode (Hererodera avenae) by scannin electron microscopy. Can J Microbiol. https://doi.org/10.1139/m88-101

Marini-Macouzet C, Muñoz L, Gonzalez-Rubio A, Eguiarte LE, Souza V, Velez P (2020) Experimental analysis of interactions among saprotrophic fungi from a phosphorous-poor desert oasis in the chihuahuan desert. Mycobiology. https://doi.org/10.1080/12298093.2020.1788271

McInroy JA, Kloepper JW (2007) Studies on indigenous endophytic bacteria of sweet corn and cotton. Molecular ecology of rhizosphere microorganisms: biotechnology and the release of GMOs. Wiley. https://doi.org/10.1002/9783527615810.ch2

Pal KK, Gardener BMS (2006) Biological control of plant pathogens. Plant Health Instr. https://doi.org/10.1094/PHI-A-2006-1117-02

Rajkumar M, Lee WH, Lee KJ (2005) Screening of bacterial antagonists for biological control of Phytophthora blight of pepper. J Basic Microbiol 45:55–63

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol. https://doi.org/10.1111/j.1469-8137.2009.02773.x

Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: A source of novel biologically active secondary metabolites. Mycol Res. https://doi.org/10.1017/S0953756202006342

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol Econ. https://doi.org/10.1016/S0168-6496(01)00189-1

Shishido M, Breuil C, Chanway CP (1999) Endophytic colonization of spruce by plant growth-promoting rhizobacteria. FEMS Microbiol Econ. https://doi.org/10.1016/S0168-6496(99)00011-2

Soliman SSM, Greenwood JS, Bombarely A, Mueller LA, Tsao R, Mosser DD, Raizada MN (2015) An endophyte constructs fungicide-containing extracellular barriers for its host plant. Curr Biol. https://doi.org/10.1016/j.cub.2015.08.027

Spadaro D, Gullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Prot. https://doi.org/10.1016/j.cropro.2004.11.003

Steward GA (2011) Growth and yield of New Zealand kauri. University of Canterbury, New Zealand (Master's thesis)

Stone JK, Bacon CW, White JF (2000) An overview of endophytic microbes: endophytism defined. Microbial Endophytes. https://doi.org/10.1163/_q3_SIM_00374

Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. A Soil Eco. https://doi.org/10.1016/S0929-1393(00)00094-9

Tan RX, Zou WX (2001) Endophytes a rich source of fuctional metabolites. Nat Prod Rep 18:448–459

Tellenbach C, Sumarah MW, Grünig CR, Miller JD (2013) Inhibition of Phytophthora species by secondary metabolites produced by the dark septate endophyte Phialocephala europaea. Fungal Ecol. https://doi.org/10.1016/J.FUNECO.2012.10.003

Terhonen E, Sipari N, Asiegbu FO (2016) Inhibition of phytopathogens by fungal root endophytes of Norway spruce. Biol Control. https://doi.org/10.1016/j.biocontrol.2016.04.006

Tuite J (1969) Plant pathological methods: Fungi and bacteria. Burgess Pub. co., Minneapolis, Minn, p 239

Velez P, Espinosa-Asuar L, Figueroa M, Gasca-Pineda J, Aguirre-von-Wobeser E, Eguiarte LE, Hernandez-Monroy A, Souza V (2018) Nutrient dependent cross-kingdom interactions: Fungi and bacteria from an oligotrophic desert oasis. Front Microbiol. https://doi.org/10.3389/fmicb.2018.01755

Waipara NW, Hill S, Hill LMW, Hough EG, Horner IJ (2013) Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. N Z Plant Prot 66:235–241

Weir BS, Paderes EP, Anand N, Uchida JY, Pennycook SR, Bellgard SE, Beever RE (2015) A taxonomic revision of Phytophthora Clade 5 including two new species, Phytophthora agathidicida and P. cocois. Phytotaxa. https://doi.org/10.11646/phytotaxa.205.1.2

Zahid S, Udenigwe CC, Ata A, Eze MO, Segstro EP, Holloway P (2006) New bioactive natural products from Coprinus micaceus. Nat Prod Res. https://doi.org/10.1080/14786410601101829

Zhan A, Schneider H, Lynch JP (2015) Reduced lateral root branching density improves drought tolerance in maize. Plant Physiol. https://doi.org/10.1104/pp.15.00187

Ziadi N, Zebarth BJ, Bélanger G, Cambouris AN, Xu G, Fan X, Miller AJ, Wang CC, Wang X, Van Nostrand JD, Deng Y, Xiaotao L, Zhou J, Han X, Deshwal VK, Singh SB, Kumar P, Chubey A, Vk D, Ryu CM (2016) Endophytic bacteria in microbial preparations that improve plant development (review). Front Microbiol. https://doi.org/10.1007/s11104-015-2778-9

Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiol Rev. https://doi.org/10.1111/j.1574-6976.2008.00123.x

Acknowledgements

The research team acknowledges Te Kawerau ā Maki and the support of Kauri Ora Community for our research on kauri, a taonga species in Aotearoa. The authors would like to thank Bella Burgess for assisting in the NCBI data submission, and Diana Lee for her technical help in culturing the endophyte isolates.