



Effects of fungicides on the beneficial seed-borne microbiome and seedling development of a long-lived myrtaceae tree species

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Abstract

Seeds are often treated with fungicides and antibiotics to eliminate pathogens during propagation. However, seeds harbour complex microbial communities, including beneficial endophytes that could also be affected by seed treatments. *Metrosideros excelsa* (Myrtaceae) is a New Zealand endemic tree widely planted within the country and overseas. Seedlings of *M. excelsa* display wide variation in susceptibility to a fungal pathogen *Austropuccinia psidii*, which causes myrtle rust disease suggesting that seed-borne microbial composition may play a role in seedling protection and growth. We investigated the effects of fungicides on the beneficial seed-borne bacterial isolates, the effects of antimicrobial treatment on seedling development, the plant growth-promoting properties of the seed microbiome, and the ability of bacterial isolates to suppress pathogenic fungi. Our results show that seed-borne bacteria *Bacillus* and *Priestia* suppressed seed-borne fungi, both pathogenic and endophytic, with average inhibition rates of 74%, and several bacterial isolates reduced *A. psidii* spore germination *in vitro*. In contrast, endophytic *Methylobacterium* and *Mycolicibacterium* facilitated fungal growth. Fungicide applications are considered the most effective method to control myrtle rust disease; however, we found that fungicides may adversely impact beneficial seed-borne bacteria. Antimicrobial seed treatment also suppressed the growth of corresponding seedlings. Our experiments demonstrate that treated seeds can be further inoculated with beneficial seed-borne endophytes, increasing seedling shoot biomass up to three times. Additionally, the bacterium *Kocuria* and the fungus *Penicillium* significantly enhanced root development. These results suggest that employing seed-borne microbial isolates with growth-promoting potential may improve outcomes in nursery conditions.

Keywords Biological control · Plant growth promotion · Pōhutukawa · Seed inoculation · Tree endophytes

1 Introduction

Seeds are occupied by diverse microbial communities (Verma and White 2019; Franić et al. 2020). Although host-specific, seed-borne fungal communities may differ among individual trees within a single population and these differences are retained within seeds after dispersal (Fort et al. 2021; Alvarado et al. 2024). Endophytic bacteria can

influence fungal assemblages by suppressing certain fungi and facilitating the growth of other fungal taxa isolated from the same plant (Christian et al. 2021; Newcombe et al. 2023). These seed-borne microorganisms can be vertically transmitted to seedlings and be founders of their endophytic microbiome (White et al. 2019).

The bacteria and fungi accumulated in seeds can be beneficial, contributing to protection against phytopathogens and drought resistance, influencing dormancy, germination rate, and seedling survival, or contributing to the invasive potential of weedy species (Chee-Sanford et al. 2006; Goggin et al. 2015; Nelson 2017; Jeong et al. 2021). Seeds can also accumulate pathogenic microorganisms that cause germination failure, seedling mortality, or, in the case of vertically transmitted latent pathogens, abnormal development of seedlings, a lower growth rate following germination and higher susceptibility to unfavourable environmental factors (Lupo et al. 2001; Mangwende et al. 2020a; Fort et al. 2021).

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Treatments to suppress these pathogens can also interfere with microorganisms providing beneficial outcomes.

As seeds may contain phytopathogens, in commercial production settings they are treated with antibiotics and fungicides to eliminate bacteria and fungi and in this way protect germinating seedlings (Fulkerson and Tossell 1955; Mancini and Romanazzi 2014; Moumni et al. 2023). Although repetitive treatment with synthetic chemicals is considered to be the most effective method for management of seed-borne pathogens, antimicrobial chemical compounds can adversely impact seedling development, especially root formation, which is crucial for the establishment of healthy plants (Hillis et al. 2011; Minden et al. 2017; Bellino et al. 2018).

Research on the effects of antimicrobial treatments on seed microbiomes is scarce, and only a few reports on the influence of antibiotics on seed bacteria exist, e.g., in *Pachycereus pringlei* (cardon cactus) (Puente et al. 2009). In a recent study, Vasanthakumari et al. (2018) found that the seed treatment with fungicides reduced the diversity of the seed-borne endophytic fungi and suppressed the development of rice seedlings, but inoculating treated seedlings with a fungal consortium recovered seedling growth. Yadav et al. (2022) demonstrated that the systemic fungicide propiconazole significantly decreased the population of endophytic bacteria in tomato plants, making them more susceptible to bacterial speck disease; however, the inoculation of fungicide-resistant *Bacillus subtilis* together with fungicide treatment induced systemic resistance to the disease. Similarly, seed coating with fungicides and spores of selected fungicide-resistant fungi improved barley and oat seedling survival and development (Murphy et al. 2016). Such data on the sensitivity of seed-associated microbiomes to widely applied agrochemicals is fundamental to understanding the deleterious effects on seedlings from the loss of a functional seed microbiome. Sustainable plant disease management may require a shift from synthetic chemical compounds to practices maximising the benefits accruing from the application of natural microbial communities that enhance seedling success.

Our research explores the sensitivity of the seed-borne microbiome of *Metrosideros excelsa* (Myrtaceae; pōhutukawa) to antibiotics and synthetic fungicides. *Metrosideros excelsa* is a New Zealand endemic tree naturally distributed on sea cliffs or volcanic rocks in the North Island of New Zealand; it is extensively planted around the country and overseas (Simpson 2005; Bylsma et al. 2014). Myrtle rust disease caused by *Austropuccinia psidii*, a recently arrived fungal pathogen, poses a threat to New Zealand Myrtaceae, and trees planted in urban areas are especially vulnerable to the infection, albeit with wide variation in susceptibility (Sutherland et al. 2020; Toome-Heller et al.

2020). Experimental inoculations of *M. excelsa* with urediniospores (asexual spores) of *A. psidii* demonstrated that seedling resistance is low, as only one plant was resistant out of the 570 tested (Smith et al. 2020). However, New Zealand sourced seeds for that study were treated with antimicrobial solutions before being propagated in a greenhouse in a potting mix with fertilisers (Smith et al. 2020). This seed treatment may have eliminated the beneficial seed microbiome, including taxa that enable natural protection against phytopathogens (Irizarry and White 2017; Sharma et al. 2022; Andreolli et al. 2023). There are limited data on the effects of the seed microbiome of Myrtaceae on seedling development or whether any microorganisms occupy plant tissues after seed treatment with antimicrobial solutions, and little is known about the impact of fungicides on beneficial seed-borne bacteria, which may naturally suppress fungal pathogens and enhance seedling development.

To address these research gaps, we explored the effects of fungicides on the beneficial seed-borne bacterial isolates, the effects of antimicrobial treatment on seedling development, plant growth-promoting properties of the seed microbiome, and the ability of bacterial isolates to suppress pathogenic fungi. We examined (i) the in vitro interactions between beneficial seed-borne bacteria and both beneficial and phytopathogenic seed-borne fungi, (ii) the inhibition effects of bacterial isolates on *A. psidii* spore germination, (iii) the sensitivity of isolated bacteria to synthetic fungicides that were effective against myrtle rust in trials, (iv) the effects of antimicrobial treatment on seed germination and development of *M. excelsa* seedlings, and (v) whether it is possible to inoculate the beneficial seed-borne microorganisms into treated seeds to reverse the adverse effects of antimicrobial solutions.

2 Materials and methods

2.1 Plant material and selection of seed-borne isolates for further experiments

To examine the role of antimicrobial compounds, we used *Metrosideros excelsa* seeds aseptically extracted from closed seed capsules to avoid environmental microbial contamination. The seed capsules were collected in March–April 2022 from 30 trees in the Auckland region, North Island, New Zealand (Table S1). The isolation of bacteria and fungi from *M. excelsa* seeds was carried out using the protocol described in our previous study (Kholostiaikov et al. 2024). Briefly, closed seed capsules with ripe seeds were surface sterilised (30 s in 70% ethanol, 2 min in 2% sodium hypochlorite, and a double wash in sterile water for 2 min each time); afterwards, seed capsules were dipped in ethanol

and left in a laminar hood for one hour. Seeds from 30 seed capsules of each tree were extracted with a sterile scalpel, pooled together, and representative bulk samples from individual trees were stored in separate containers at 4 °C.

Approximately 100 viable seeds from each bulk sample were soaked in sterile water overnight in individual Petri dishes and crushed with a pestle and mortar. A sterile leaf infusion (20 g of fresh *M. excelsa* leaves autoclaved in 0.5 L of sterile water) was added to the crushed seeds, which were then placed onto Petri dishes containing five types of media: PDA, R2A, YNA (Difco, Becton, Dickinson, and Company), M9 (Sigma-Aldrich), and M9 with methanol, respectively, and incubated for at least two months at an approximate temperature of 18–20 °C. The employment of various media and host leaf infusions allowed the isolation of a more comprehensive taxonomic range of seed microorganisms (Eevers et al. 2015; Sarhan et al. 2019).

Bacterial 16 S and fungal ITS gene regions of emergent colonies were sequenced as described in (Kholostiakov et al. 2024), and, based on the genetic identities of the isolates, their biocontrol potential and growth-promoting activity were catalogued using available literature. Fourteen bacterial isolates were selected as putative beneficial endophytes, with the literature describing growth-promoting properties and antagonism towards phytopathogenic fungi (Table 1). These bacteria were retained for dual culture assays with fungi and seed inoculation experiments (Table 1). Bacteria, reported as potential plant or human pathogens, were excluded from the experiments, except *Pantoea* sp., whose 1350 bp DNA fragment of the 16 S rRNA indicated a close similarity to *Pantoea ananatis*, but it was included in experiments due to the reported high genetic and functional variability of its strains in plant hosts, ranging from highly pathogenic strains to enhancing plant growth and improving stress tolerance (Sheibani-Tezerji et al. 2015; Weller-Stuart et al. 2017; Lu et al. 2021).

2.2 Dual culture assay

The dual culture spot inoculation method was employed to evaluate the interactions of isolated bacteria and fungi (Qi et al. 2021). For this assay, 14 bacterial isolates with beneficial properties and 12 fungal isolates, both beneficial and pathogenic, were used (Table 1). To account for some taxa growing faster on certain media, we employed Waksman agar (WA, Difco, Becton, Dickinson, and Company) for the assays with the four bacterial isolates (*Kocuria* sp., *Paenibacillus* sp., *Peribacillus* sp., and *Priestia* sp.), and potato dextrose agar (PDA, Difco, Becton, Dickinson, and Company) for the remaining isolates. The bacterial cultures were streaked equidistantly on PDA and WA plates and allowed to grow overnight, then the hyphal tips of fungal mycelium

were placed in the centre of each Petri dish (Fig. 1). As *Methylobacterium* is a slow-growing genus, we followed the methodology from a recent study (Christian et al. 2021) and allowed the bacterial colonies to become established on media for four days before fungi were introduced. All inoculations were done in triplicate, and three plates with the corresponding fungus without bacteria were used as a control.

The plates were incubated at 24 °C, examined daily, and measured when fungal colonies in control plates occupied $\geq 80\%$ of the surface. The diameter of the fungal mycelia on the test and control plates was measured to determine the antagonistic effect. The inhibition was calculated as a percentage of fungal colony diameter with bacteria relative to controls following the formula: % inhibition = $(1 - (\text{Fungal colony diameter with bacteria} / \text{Control diameter}) \times 100\%$ (Montealegre et al. 2003; Newcombe et al. 2023). Inhibition was recorded as a negative value, and when the diameter of the fungal colony was larger in the presence of bacteria, this was reflected as a positive value (del Barrio-Duque et al. 2019; Newcombe et al. 2023). When the fungal growth in dual culture was decreased or increased $\leq 10\%$ compared to the control, it was considered a neutral interaction and recorded as 0 in a corresponding table of results.

2.3 *Austropuccinia psidii* urediniospore germination assay

This bioassay was performed following the protocol developed for spore germination assay at Plant and Food Research, Lincoln (Chng, pers. com.). Bacterial strains used in this experiment are listed in the Table 1. A fresh bacterial colony was inoculated in 2.5 mL of Luria Bertani broth (Difco, Becton, Dickinson, and Company) and incubated at 24 °C overnight with shaking (300 rpm, Eppendorf Thermo-Mixer® C). The following day, 1 mL of grown culture was centrifuged at 15,000 g for 5 min. To determine bacterial cell density, the pellet was re-suspended in 1 mL of 0.5% M9 salts (Sigma Aldrich) and optical density at 600 nm was measured. Bacterial suspensions were diluted in 0.5% M9 salts at a final $OD_{A600} = 0.1$.

Water-agar slides were prepared by adding 2 mL of a sterile solution of 2% water agar on top of plain glass microscope slides in a horizontal position to form a thin agar layer. After solidifying, water-agar slides were placed into 100 × 100 mm square Petri dishes, three per plate, and covered. Slides were kept at 4 °C under humid and sterile conditions until the experiment.

For rust inoculation, freshly collected urediniospores of *A. psidii* were suspended in light mineral oil for cell culture (M8410, Sigma Aldrich) just prior to inoculation onto the glass slides (Yong et al. 2018). Petri dishes containing

Table 1 Isolates used for dual culture and urediniospore germination assays. Bacterial isolates employed for seed inoculation experiment are highlighted in bold. Abbreviations OTH3, U5, etc., after organism name reflect a source tree of the isolate (Table S1). Sequence identity reflects the percentage of the alignment of 1340–1360 bp fragment of 16 S rRNA gene sequence for bacteria and, for fungi, a 580–590 bp sequence of ITS1-ITS2 region of 5.8 S rRNA gene against sequences provided by NCBI. All isolates were deposited in international collection of microorganisms from plants (ICMP) and can be requested for use

Organism	The closest taxon (GenBank accession number)	Sequence identity (%)	GenBank accession number	ICMP number	Putative functions in plant hosts, based on literature records	Reference
Bacteria						
<i>Bacillus mycoi-</i> <i>des</i> TD3	<i>B. mycoides</i> (MN519467)	100	OR472920	24867	Systemic pathogen resistance, nitrogen fixation	Bargabus et al. 2002; Singh et al. 2020; Kurniawan and Chuang 2022
<i>Bacillus</i> sp. OTH3	<i>B. amyloliquefaciens</i> (CP054415)	100	OR472917	25417	Nutrient acquisition, plant growth-promoting, pathogen resistance	Wang et al. 2020; Luo et al. 2022
<i>Bacillus</i> sp. U5	<i>B. velezensis</i> (MH177236)	98.89	OR472918	25418		
<i>Bacillus</i> sp. W2	<i>B. stercoris</i> (PP257415); <i>B. subtilis</i> (ON778661)	99.75	OR472921	25419	Growth-promotion, salt and heat stress reduction	War Nongkhla and Joshi 2014; Xu et al. 2014; Yousuf et al. 2017; Bokhari et al. 2019; Hashem et al. 2019; Wang et al. 2021
<i>Bacillus</i> sp. OTH5	<i>B. amyloliquefaciens</i> (PP572911); <i>B. tequilensis</i> (PP563728)	99.86	PP694398	25420	Growth-promotion, pathogen resistance	Bhattacharya et al. 2019
<i>Kocuria</i> sp. TD1	<i>K. palustris</i> (MT534060)	100	PP694319	24,865	Growth-promotion, nutrient acquisition, salt-tolerance	Zacaria Vital et al. 2019; Wang et al. 2020; Afridi et al. 2021
<i>Methylobacterium</i> sp. U4	<i>Methylobacterium</i> sp. (MT360236)	100	PP716426	24861	Pathogen resistance, growth-promotion, nutrient acquisition, facilitation of beneficial fungal endophyte	Ardanov et al. 2012; Madhaiyan et al. 2015; del Barrio-Duque Alejandro et al. 2020; Senthil Kumar et al. 2021
<i>Methylobacterium</i> sp. Tw3	<i>M. phyllostachyos</i> (NR_108242)	99.86	PP716427	24863		
<i>Mycolicibacterium</i> sp. U5	<i>M. hippocampi</i> (MW012620)	99.7	OR472927	24860	Pathogen resistance, facilitation of beneficial endophyte	del Barrio-Duque et al. 2019
<i>Paenibacillus</i> sp. Tw2	<i>Paenibacillus</i> sp. (KF465973)	99.78	PP694321	25421	Growth-promotion, pathogen antagonism, nutrient acquisition	Rybakova et al. 2016; del Barrio-Duque et al. 2019; Liu et al. 2019
<i>Pantoea</i> sp. TD1	<i>P. ananatis</i> (MG574389)	100	PP702021	24857	Depending on species, saprophytes, pathogens, or beneficial endophytes	Sheibani-Tezerji et al. 2015; Weller-Stuart et al. 2017; Lu et al. 2021; Bragard et al. 2023
<i>Pantoea</i> sp. OTH1	<i>P. allii</i> (OM471718)	100	OR472915	25102		
<i>Peribacillus</i> sp. TD1	<i>P. frigoritolerans</i> (PP623087)	99.93	PP694317	24859	Growth-promotion, nutrient acquisition, salt-tolerance	Meena et al. 2017; Wang et al. 2020; Egamberdieva et al. 2021; Li et al. 2021
<i>Priestia</i> sp. U3	<i>P. megaterium</i> (PP528572); <i>P. aryabhattai</i> (PP470985)	99.93	PP694320	24858	Growth-promotion, nutrient acquisition, systemic pathogen resistance	Park et al. 2017; Deng et al. 2022; Shahid et al. 2022
Fungi						
<i>Alternaria</i> sp. Tw1	<i>A. tenuissima</i> (OQ507771); <i>A. alternata</i> (PP065528)	100	PP702004	25386	Pathogen or endophyte	Crous et al. 2019; DeMers 2022; Elghaffar et al. 2022
<i>Aureobasidium</i> sp. W2	<i>Aureobasidium</i> sp. (OK376894)	99.81	PP702007	24847	Broad spectrum biocontrol agent, used in seed coating	Wachowska et al. 2016; Di Francesco et al. 2020;

Table 1 (continued)

Organism	The closest taxon (GenBank accession number)	Sequence identity (%)	GenBank accession number	ICMP number	Putative functions in plant hosts, based on literature records	Reference
<i>Cladosporium</i> sp. W3	<i>C. sp.</i> (PP151182); <i>C. cladosporioides</i> (PP097797)	99.82	PP702008	25393	Endophytes, some species can be rust mycoparasites	Kohl et al. 2015; Qin et al. 2016; Torres et al. 2017; da Silva et al. 2023
<i>Cladosporium</i> sp. W5	<i>C. halotolerans</i> (MN859971)	100	PP702009	25392		
<i>Cladosporium</i> sp. U3	<i>C. delicatulum</i> (MT548673); <i>C. peran-gustum</i> (MT466522)	100	PP702010	25394		
<i>Colletotrichum</i> aff. <i>florinae</i> TD2	<i>C. schimae</i> (MZ595887)	100	PP702000	25387	Endophyte or pathogen	Peres et al. 2005; Mangwende et al. 2020b; Martin and Peter 2021
<i>Cytospora</i> sp. OTH4	<i>C. pruinosa</i> (EU552121)	99.32	PP702002	25388	Tree pathogens	Lupo et al. 2001; Adams et al. 2005; Crous et al. 2019; Pan et al. 2020
<i>Cytospora</i> sp. W5	<i>C. sp.</i> (OR499136)	100	PP702003	24850		
<i>Neofusicoccum</i> aff. <i>luteum</i> W1	<i>N. luteum</i> (MH183385)	100	PP701999	24852	Pathogen	Sergeeva et al. 2009; Shetty et al. 2011; Hanin and Fitriarsi 2019; Mangwende et al. 2020a; Liu et al. 2022; Waqas et al. 2022
<i>Neopestalotiopsis</i> sp. Tw3	<i>N. clavisporea</i> (MN519192); <i>N. rosae</i> (PP430826)	99.82	PP702005	25390	Endophyte or pathogen	Maharachchikumbura et al. 2014; Reddy et al. 2016; Prasan-nath et al. 2021
<i>Penicillium</i> sp. Sh3	<i>P. coffeae</i> (MT309664); <i>P. olsonii</i> (MG252481)	100	PP702001	25389	Saprophyte or endophyte, enhance nutrient acquisition and stress tolerance	Yamaji et al. 2001; Doilom et al. 2020; Molina-Montenegro et al. 2020; Ismail et al. 2021
<i>Pestalotiopsis</i> sp. U5	<i>Pestalotiopsis</i> sp. (KP689121)	99.84	PP702006	25391	Depending on species, pathogens or endophytic antagonists of pathogens	Maharachchikumbura et al. 2014; González and Estefanía 2020

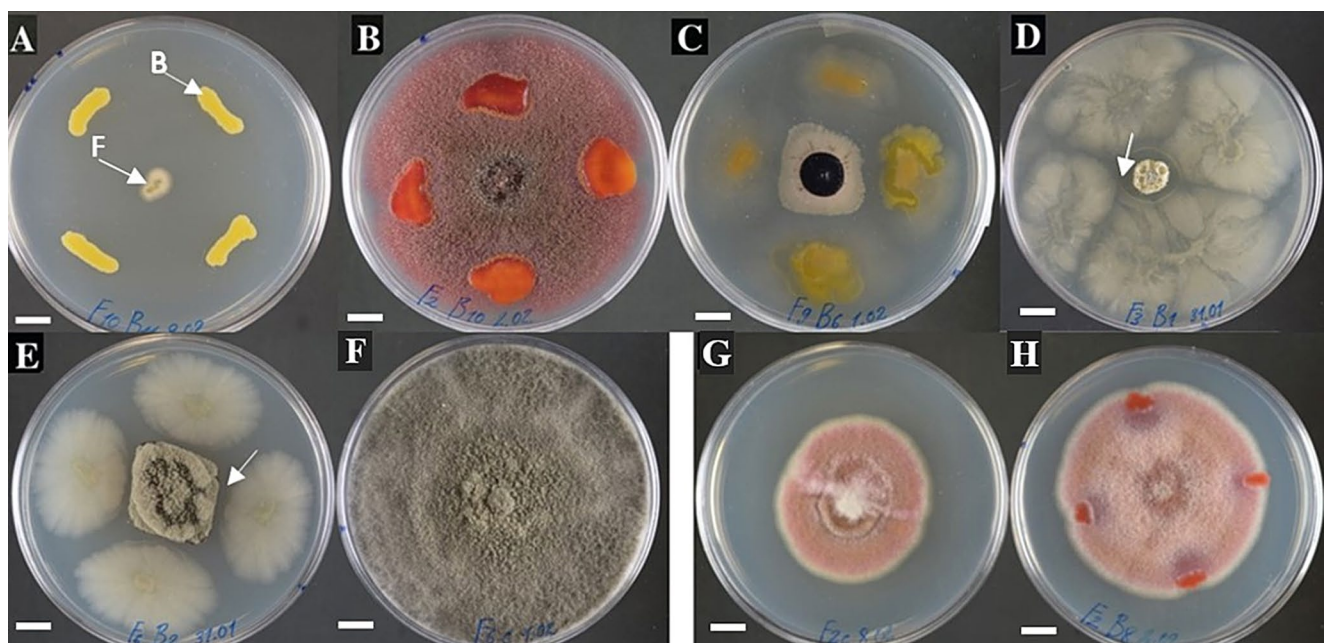


Fig. 1 Dual culture interaction assay. (A) Plate on the second day after inoculation, arrows show position of B- bacteria and F- fungus, respectively. (B) Fungal growth is not inhibited by *Methylobacterium* sp. (C) *Pantoea* sp. demonstrates strong antagonistic activity towards *Aureobasidium* sp. (D) *Bacillus* sp. suppressed growth of *Penicillium*

sp., arrow at the gap between bacterial and fungal colony. (E) inhibition of *Alternaria* sp. by *Bacillus* sp. and *Alternaria* sp. control (F). (G) *Colletotrichum* aff. *florinae* control, and *Methylobacterium* sp. facilitates the growth of *C. aff. florinae* (H). Scale bar = 1 cm

water-agar slides were opened, and spores were spread onto slides using a sterilised paintbrush. Inoculated slides were left drying, uncovered for 60 min in a laminar flow until the spores settled into agar.

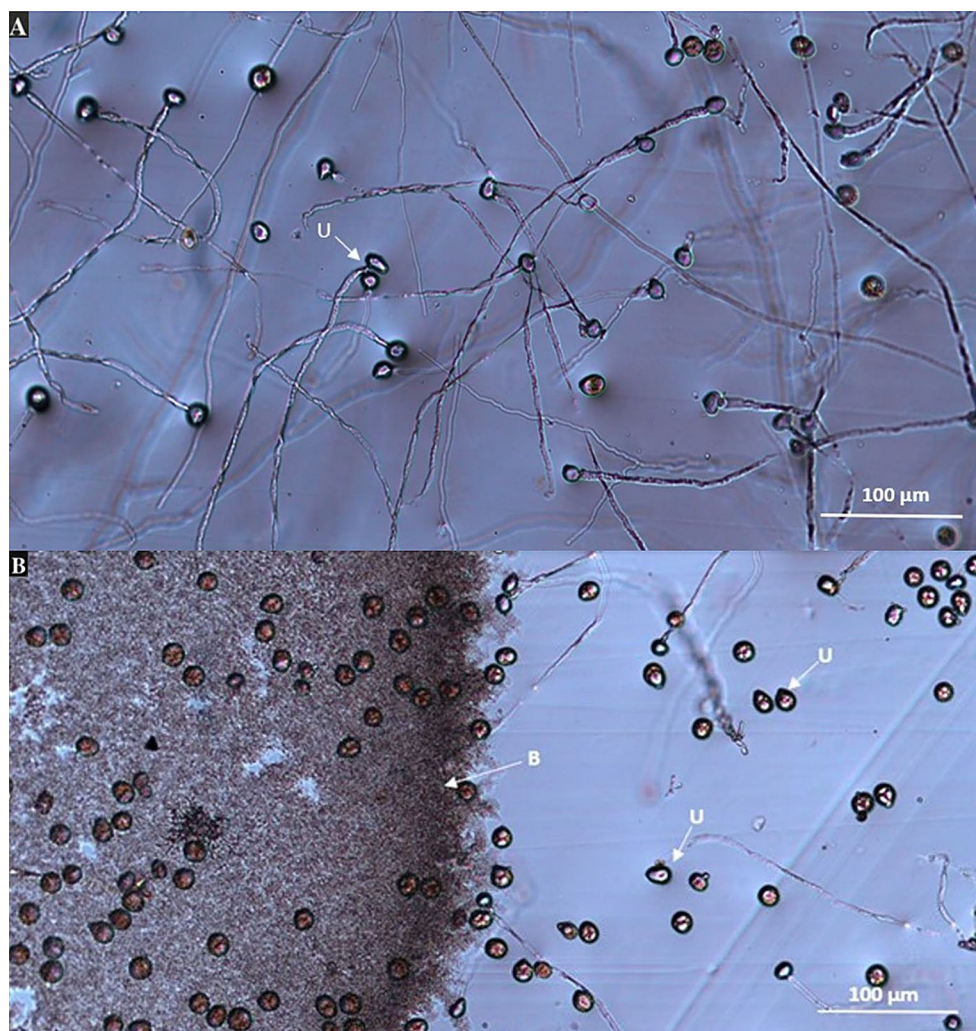
To analyse the effect of bacterial cells, 20 mL of bacterial suspension was inoculated at three separate points on each slide with *A. psidii* spores from a Petri dish (three slides), which resulted in nine dots of bacterial cells per sample. Plates were covered and incubated at 20 °C under dark and humid conditions for 24 h. As a positive control, three slides with spores were inoculated with 20 mL of 0.5% M9 salts, and another three slides sprayed with a fungicide, Plant Health Fungus Control (Kiwicare, New Zealand) as a negative control. After the incubation, slides were exposed to UV for 30 min to kill viable spores, then one field per dot was counted under 200× magnification, and the germination rate was determined as follows: $GR = \text{rust spores germinated} / \text{total number of spores} \times 100$ (Fig. 2).

2.4 Fungicide sensitivity of bacteria

This test was carried out on the same bacterial isolates used in 2.3, employing a modified method recently developed for endophytic bacteria (Andreolli et al. 2023). We used two synthetic formulations: Fungus Gun (FG, Yates, Australia) containing 0.05 g/litre myclobutanil, and Plant Health Fungus Control (PHFC, Kiwicare, New Zealand) containing boscalid 0.25 g/L, pyraclostrobin 0.13 g/L, and tebuconazole 0.43 g/L. Myclobutanil and pyraclostrobin were tested against myrtle rust by Beresford and Wright (2022) and gave excellent results in myrtle rust control trials. The compounds tested in our study target different molecular activities in fungi: boscalid and pyraclostrobin inhibit fungal respiration by blocking the ubiquinone-binding sites in the mitochondrial complex II and cytochrome b, respectively, whereas myclobutanil and tebuconazole disrupt ergosterol of fungal cell walls (Karadimos et al. 2005; Avenot and Michailides 2010; Wang et al. 2024).

To test the sensitivity of bacterial isolates, the fungicides were sprayed on PDA and WA media, and Petri dishes were

Fig. 2 Urediniospore germination on water agar (above) and in the presence of *Bacillus* sp. U5 (below); white arrows: U—urediniospores, and B—bacterial colony. While most of urediniospores produced long germination tubes on water agar, they could not germinate with *Bacillus* sp. U5. Scale=100 µm



allowed to dry in the laminar hood for around 30 min to evaporate excessive moisture. Then, bacteria were streaked on plates with and without fungicides. Assays were conducted in triplicates. Petri dishes were incubated in the dark at 24 °C for six days, and the inhibition of bacterial growth was recorded when the colony diameter was reduced by more than 50% with respect to control after six days of incubation.

2.5 Trial on the influence of antimicrobial solutions on seed germination and culturable endophytic microbiome of seedlings

To evaluate the seed germination rate after extended storage, the seed and seedling sensitivity to antimicrobial solutions, and the presence of a microbiome in seedlings after treatment, an initial trial was carried out on seeds collected from three individual trees located in well separated populations (Trees 1–3, Table S1), that had been stored in sterile containers at 4 °C for more than one year. Seeds from each tree were soaked for three days in antibiotic antimycotic solution (62.5 µg/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B in citrate buffer) (Sigma Aldrich, USA) recently used for the in-vitro propagation of another New Zealand native Myrtaceae species (van der Walt et al. 2023). Control seed groups were soaked in sterile water (Fig. 3). The germinating seeds were sprayed with the fungicide PHFC to remove any residual endophytes.

Approximately 10–18% of *M. excels* seeds are fertile; the mean weight of the fertile seed is 0.15 mg and 0.1 mg for infertile (Schmidt Adam et al. 1999, 2002). Therefore, we roughly estimated number of viable seeds by weight and soaked 120 mg of seeds from each tree separately in eight Petri dishes, four control and four with antimicrobial solution. Then seeds from two control dishes and two treated were placed on water agar (Difco, Becton, Dickinson, and

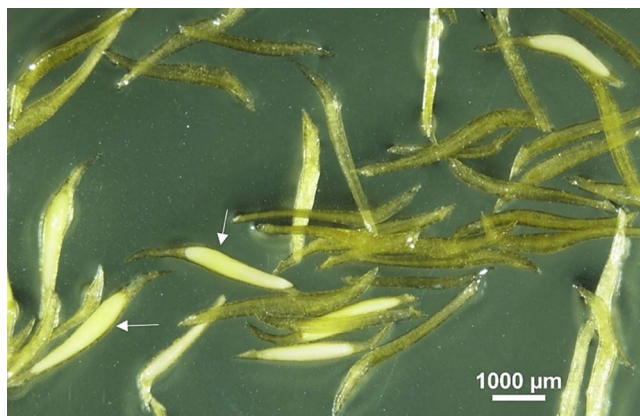


Fig. 3 *Metrosideros excelsa* seeds. Growing embryos (arrows) are visible in filled seeds after three days in water; up to 90% of seeds do not contain embryos. Scale bar = 1000 µm

Company), and seeds groups from another four dishes (two control and two treatment) were sown in sterile 473 mL containers for plant tissue culture (PhytoTech Labs, USA). The containers were half-filled with potting mix (fine-grade pumice/coir peat/topsoil in ratio 50/40/10 by volume) that had autoclaved once as a bulk soil mix and then three times within the containers for three consecutive days to ensure sterility. To check sterilisation efficiency, the potting mix was plated on PDA and yeast nutrient agar (YNA, Difco, Becton, Dickinson, and Company) and observed for two weeks.

Containers and Petri dishes with seeds were placed in a growth chamber under 12 h daylight and 12 h dark under full spectrum LED horticultural light (Growshop, New Zealand) and day/night temperatures 25–26 °C/19–21 °C. Emergent seedlings were counted, and their development was observed under a microscope every day for one week. One month after germination, ten random seedlings from each group were removed, sterilised by immersion in 70% ethanol for 30 s, then transferred to 2% sodium hypochlorite for 2 min, followed by a double wash in sterile water for 2 min each time, and plated on PDA and YNA. Roots and shoots were plated separately to assess any difference in the culturable microbiome within these organs. Emergent colonies were sequenced as described in (Kholostiaikov et al. 2024). The sequences of the isolates are available in the GenBank NCBI under accession numbers PP694316, PP694392–PP694397, OR499151–OR499152, PP708936–PP708938.

2.6 Seed inoculation experiment

To assess the influence of the culturable seed microbiome on the growth of *M. excelsa* seedlings, nine bacterial isolates were chosen for seed inoculation (Table 1). Isolates were selected based on the results of pilot experiments and literature records on the beneficial properties of endophytes (Table 1). *Penicillium* sp. W5 isolated from Tree 3 seeds during the seed germination trial (Sect. 2.5) was also employed in the seed inoculation experiment.

Seeds collected from Tree 1 (Table S1) were soaked for six days in the antibiotic antimycotic solution in 12 small Petri dishes, 30 mg of seeds in each, corresponding to 12 treatment applications: nine seed groups were inoculated with single bacterial isolate (Table 1), one seed group was inoculated with consortium of all nine bacterial isolates, one - with *Penicillium* sp. W5 isolated from seedlings during the germination trial (2.5), and one seed group was a treated control without subsequent inoculation.

After the treatment with the antibiotic antimycotic solution, seeds were immersed in fungicide PHFC for 6 h, washed twice in sterile water, left in plates for 48 h, then placed in the microbial inoculum overnight. For the

inoculum, isolates of *Bacillus* and *Methylobacterium* were added to 2.5 mL of Potato Dextrose Broth (Difco, Becton, Dickinson, and Company) and all other isolates were placed in Nutrient Broth (Difco, Becton, Dickinson, and Company). The microbes were incubated at 25 °C for 24 h with shaking (300 rpm, Eppendorf ThermoMixer® C), centrifuged at $4000 \times g$ for 10 min, then resuspended in sterile water to the concentration 10^3 – 10^5 CFU/ml, and 2 ml of these cell suspensions were added to germinating seeds in Petri dishes. One group of seeds was immersed in a bacterial consortium created by mixing cell suspensions of all nine bacterial isolates in equal volumes. Spores of *Penicillium* sp. W5 were washed from fresh cultures on PDA and spore suspension was adjusted to 1×10^5 cells/ml. The next day after inoculation in Petri dishes, seeds were planted on the sterilised substrate in containers (prepared as described in Sect. 2.5), at least ten seeds per container. At this stage, germinating seeds had already begun photosynthesis, making possible to separate green viable seeds from non-viable ones. In parallel with treatment and inoculation procedures, 30 mg of untreated seeds from the same Tree 1 were germinated in autoclaved water and sown in sterilised containers as a positive control. All operations with seeds and emerging seedlings were conducted in a laminar hood. Inoculations and control were performed in triplicates.

Containers were sealed for three months and placed in a growth chamber (light and temperature conditions as in 2.5); containers were randomly rotated around the chamber weekly to ensure that light variation due to edge effects did not influence seedling development. Seedlings were counted after two weeks; if there were more than 12 seedlings, the extra seedlings were randomly removed, and containers were sealed until the end of the experiment. After three months, containers were opened, and seedlings were washed in water. Seedling growth parameters were measured as follows: root length (mm); shoot height (mm); number of shoot branches; number of leaf pairs; fresh weight of shoots; and root branching (1– only one primary root with small branches, 2– two or three main roots, 3– more than three main roots with extensive branching). Root weight was not measured because *M. excelsa* seedlings have fine roots, which were covered with particles of potting mix that were impossible to remove without damaging the roots.

2.7 Recovery of inoculated taxa through a culture-based approach

Roots and shoots of five random seedlings from each container were cut and sterilised separately from each other in 70% ethanol for two minutes, 2% sodium hypochlorite solution for two minutes, and a double wash in sterile water for two minutes each time. Then 3–4 mm long pieces of

shoots and roots were placed on PDA, R2A, and M9 agar with 0.5% methanol and observed daily for one month. The sequences of the isolates are available in the GenBank NCBI under accession numbers OR472927, PP702015–PP702030. The pure cultures of the isolates were deposited in ICMP, Manaaki Whenua– Landcare Research, Auckland, New Zealand, under numbers ICMP 25398–25416.

2.8 Statistical analysis

The difference in seed germination of the treated and control groups was assessed by Welch's *t*-test. *Austropuccinia psidii* urediniospore germination rates were compared between the control and treatment groups using a one-way ANOVA and followed by Dunnett's post hoc test. For the inoculation experiment, comparisons of shoot fresh weight between the control and treatment groups were performed using a one-way ANOVA, followed by Tukey's multiple comparisons test. Other plant growth parameters after the inoculation experiment (root length, root branching, shoot height, shoot branching, and number of leaf pairs) were analysed by a two-way ANOVA to evaluate variation between seedlings from individual containers of each triplicate and determine the significance of treatment effects, followed by Tukey's multiple comparisons test. A significance level of $P \leq 0.05$ was considered statistically significant for all tests. Analyses were performed in GraphPad Prism 10.2.2 (GraphPad Software, Boston, Massachusetts, USA).

3 Results

3.1 Selection of seed-borne isolates for further experiments

According to sequencing results from pure cultures, the microorganisms isolated from *M. excelsa* seeds belonged to 16 species of bacteria and 22 fungal species (Table S2). After the literature review, 12 species of seed-borne bacteria were identified as beneficial endophytes with documented activity against phytopathogens and/or growth-promoting properties (Table 1). Phytopathogenic bacteria were represented by *Robbsia* aff. *andropogonis*. An isolate of *Pantoea* sp. closely related to *P. ananatis* was considered potentially pathogenic or beneficial, depending on the strain; therefore, it was included in further experiments to evaluate properties in vitro and *planta* (Table 1). Nine species of isolated fungi are known as biocontrol agents, while *Colletotrichum* aff. *fiorinae*, *Cytospora* sp., and *Neofusicoccum* sp. are phytopathogens of various trees, including Myrtaceae (Table 1). The rest of the isolated fungi included endophytes without any known functions. Based on the literature records, 14

bacterial strains beneficial for plant hosts were selected for dual culture assays against nine taxa of potentially beneficial endophytic fungi and three species of fungal pathogens (Table 1).

3.2 Dual culture assay

The dual culture spot inoculation method confirmed that the interactions of the seed-borne bacteria and fungi were primarily antagonistic; however, some bacteria and fungi did not suppress the growth of each other, and three bacterial isolates facilitated fungal growth (Table 2). Bacteria from the phylum Bacillota (*Bacillus*, *Peribacillus*, *Priestia*) demonstrated strong antagonism towards almost all fungal isolates, including putative plant pathogens such as *Alternaria* sp., *Colletotrichum* aff. *florinae*, *Cytospora* spp., and *Neofusicoccum* sp. (Table 2). *Neofusicoccum* aff. *luteum* was the fastest-growing species whose mycelial growth was reduced by only three bacterial isolates: *Bacillus* sp. OTH3, *Bacillus* sp. W2, and *Pantoea* sp. TD1.

Paenibacillus sp. Tw2 was the only species from Bacillota that was neutral to most of the fungal isolates. In the majority of dual culture assays with *Methylobacterium* and *Mycolicibacterium*, these bacteria established on media and were not covered in mycelium when the fungal colony reached the edge of the Petri dish, but bacteria were growing without visible antagonism towards the fungus (Fig. 1.A).

3.3 *Austropuccinia psidii* urediniospore germination assay

Several seed-borne bacteria that inhibited pathogenic fungi in dual culture assays were also able to suppress the spore germination of *A. psidii*. A bioassay on the effects of live bacterial cultures on *A. psidii* urediniospores showed a significant variation between the abilities of the seed-borne bacteria to suppress urediniospore germination (ANOVA, $P < 0.0001$; Table S3).

Bacillus sp. U5 was the most effective antagonist of the myrtle rust pathogen *in vitro*, reducing spore germination from 69.9 to 12.8% (Fig. 2; Table 3). Other members of phylum Bacillota (*Bacillus*, *Peribacillus*, and *Priestia*) demonstrated various levels of myrtle rust inhibition, except *Paenibacillus* sp., which did not affect the germination of urediniospores.

3.4 Fungicide sensitivity of bacteria

Following the assays of their antifungal activity, the same fourteen seed-borne bacterial isolates were tested against two widely available fungicides that were effective against myrtle rust in trials in New Zealand (Beresford and Wright 2022). Eight out of nine bacteria that suppressed *A. psidii* urediniospore germination *in vitro* were also sensitive to PHFC fungicide (Table 3). In contrast, only two of the *A. psidii*-antagonistic bacterial isolates, *Bacillus* sp. OTH3, and *Priestia* sp. U3, were sensitive to FG fungicide (Table 4).

Table 2 Interactions of bacteria and fungi in dual culture assay. Each cell represents a percentage of inhibition (negative values) or facilitation (**positive values**) of fungal growth in comparison to control. When the fungal growth was decreased or increased up to 10% with respect to the control, it was considered a neutral interaction and recorded as 0

	<i>Alternaria</i> sp. Tw1	<i>Aureobasidium</i> sp. W2	<i>Cladosporium</i> sp. W3	<i>Cladosporium</i> sp. W5	<i>Cladosporium</i> sp. U3	<i>Colletotrichum</i> aff. <i>florinae</i> TD2	<i>Cytospora</i> sp. OTH4	<i>Cytospora</i> sp. W5	<i>Neofusicoccum</i> aff. <i>luteum</i> W1	<i>Neopestalotiopsis</i> sp. Tw3	<i>Penicillium</i> sp. Sh3	<i>Pestalotiopsis</i> sp. U5
<i>Bacillus</i> sp. OTH3	-71	-71	-60	-58	-62	-78	-80	-95	-75	-60	-81	-58
<i>Bacillus</i> sp. U5	-69	-63	-52	-67	-62	-67	-60	-68	0	-66	-74	-50
<i>Bacillus mycoides</i> TD3	-43	-34	-47	-31	-40	-54	-82	-68	0	-44	-10	-50
<i>Bacillus</i> sp. W2	-71	-76	-72	-75	-69	-78	-93	-87	-87	-81	-67	-75
<i>Bacillus</i> sp. OTH5	-64	-58	-21	-50	-43	-35	-30	0	0	-72	-42	-50
<i>Kocuria</i> sp. TD1	0	-55	-15	0	0	0	-26	0	0	0	0	0
<i>Methylobacterium</i> sp. U4	+34	-27	+14	0	0	+18	-45	-23	0	+19	0	-24
<i>Methylobacterium</i> sp. Tw3	+21	-25	-20	0	0	0	-43	-31	0	0	0	-43
<i>Mycolicibacterium</i> sp. U5	+11	-39	0	+28	-21	0	-60	-30	0	0	0	0
<i>Paenibacillus</i> sp. Tw2	0	-45	0	-13	0	0	0	0	0	0	0	0
<i>Pantoea</i> sp. TD1	-28	-39	-70	-31	-33	-36	-11	0	-62	-95	-35	-25
<i>Pantoea</i> sp. OTH1	0	0	-25	-17	-12	-29	0	-20	0	0	0	0
<i>Peribacillus</i> sp. TD1	-39	-52	-74	-45	-53	-38	-72	-24	0	-19	-79	-31
<i>Priestia</i> sp. U3	-43	-63	-64	-40	-65	-19	-51	-40	0	0	-62	-45

Table 3 Results of assays to evaluate effects of bacterial isolates on *Austropuccinia psidii* germination and their sensitivity (neg– growth negatively affected) to two commercial fungicides, fungus gun (FG, yates, Australia) and plant health fungus control (PHFC, kiwicare, new Zealand). Urediniospore germination– values represent mean percentage of germinated spores $\pm$ standard error of the mean for nine dots with bacterial cells counted. *- indicate statistical differences between the control and bacterial isolate, assessed by Dunnett's test ($P < 0.05$)

Organism	Urediniospore germination	Fungus Gun	Plant Health Fungus Control
<i>Bacillus</i> sp. OTH3	54.5 $\pm$ 3.2*	neg	neg
<i>Bacillus</i> sp. U5	12.8 $\pm$ 2.4*		neg
<i>Bacillus mycoides</i> TD3	54.3 $\pm$ 1.3*		neg
<i>Bacillus</i> sp. W2	53.3 $\pm$ 3.8*		neg
<i>Bacillus</i> sp. OTH5	41.8 $\pm$ 2.3*		neg
<i>Kocuria</i> sp. TD1	68.6 $\pm$ 1.8	neg	neg
<i>Methylobacterium</i> sp. U4	68.3 $\pm$ 2.6		neg
<i>Methylobacterium</i> sp. Tw3	69.1 $\pm$ 2.5		neg
<i>Mycolicibacterium</i> sp. U5	53.1 $\pm$ 4.5*		neg
<i>Pantoea</i> sp. TD1	57.3 $\pm$ 2.8*		
<i>Pantoea</i> sp. OTH1	67.6 $\pm$ 1.4		
<i>Peribacillus</i> sp. TD1	49.0 $\pm$ 4.4*		neg
<i>Paenibacillus</i> sp. Tw2	70.7 $\pm$ 2.3	neg	neg
<i>Priestia</i> sp. U3	45.5 $\pm$ 2.7*	neg	
Control	69.9 $\pm$ 3.5		

Table 4 Response of *M. excelsa* seedlings to treatment with seed-borne bacteria and *Penicillium* sp. W5 after three months of growth in closed containers. Values reflect means $\pm$ standard error of the mean for 30 plants per treatment, and letters after values indicate: a– significantly different from sterilised control, b– different from water control, based on tukey's multiple comparisons test (Sup. Inf. 2)

Treatment	Root length, mm	Root branching, <i>N</i>	Shoot height, mm	Shoot branching, <i>N</i>	Leaf pairs, <i>N</i>	Shoot fresh weight, mg
Sterilised control	50.9 $\pm$ 7.1	1.5 $\pm$ 0.2 b	6.3 $\pm$ 1.1 b	1.1 $\pm$ 0.1	2.9 $\pm$ 0.6	8.0 $\pm$ 1.4
Water control	54.4 $\pm$ 4.1	2.1 $\pm$ 0.3 a	11.3 $\pm$ 1.0 a	1.0 $\pm$ 0.0	3.4 $\pm$ 0.3	12.0 $\pm$ 1.0
<i>Penicillium</i> sp. W5	63.0 $\pm$ 4.5	2.8 $\pm$ 0.1 ab	20.9 $\pm$ 1.4 ab	1.1 $\pm$ 0.1	4.8 $\pm$ 0.4 a	19.5 $\pm$ 1.3 a
<i>Bacillus</i> sp. W2	56.0 $\pm$ 5.8	2.4 $\pm$ 0.3 a	15.2 $\pm$ 2.4 a	1.5 $\pm$ 0.3	4.8 $\pm$ 0.6	23.0 $\pm$ 3.6 ab
<i>Bacillus</i> sp. U5	44.9 $\pm$ 3.4	1.8 $\pm$ 0.3	9.5 $\pm$ 1.4	1.2 $\pm$ 0.1	3.0 $\pm$ 0.4	11.0 $\pm$ 1.6
<i>Kocuria</i> sp. TD1	68.1 $\pm$ 6.1 ab	2.1 $\pm$ 0.2 a	19.3 $\pm$ 2.2 ab	1.7 $\pm$ 0.3 ab	5.5 $\pm$ 0.7 ab	34.0 $\pm$ 3.8 ab
<i>Methylobacterium</i> sp. Tw3	39.3 $\pm$ 2.6 b	2.1 $\pm$ 0.2 a	11.1 $\pm$ 1.2 a	1.4 $\pm$ 0.2	3.8 $\pm$ 0.4	7.0 $\pm$ 0.8
<i>Mycolicibacterium</i> sp. U5	52.0 $\pm$ 4.5	1.7 $\pm$ 0.2	14.5 $\pm$ 1.5 a	1.4 $\pm$ 0.2	4.9 $\pm$ 0.6 ab	23.0 $\pm$ 2.4 ab
<i>Paenibacillus</i> sp. Tw2	57.5 $\pm$ 3.9	2.2 $\pm$ 0.2 a	16.0 $\pm$ 1.2 ab	1.3 $\pm$ 0.2	4.7 $\pm$ 0.4 a	19.0 $\pm$ 1.4 a
<i>Pantoea</i> sp. OTH1	54 $\pm$ 5	1.9 $\pm$ 0.2	12.8 $\pm$ 1.3 a	1.2 $\pm$ 0.2	4 $\pm$ 0.4	15 $\pm$ 1.4
<i>Pantoea</i> sp. TD1	50.2 $\pm$ 4.5	1.9 $\pm$ 0.2	13.7 $\pm$ 1.7 a	1.4 $\pm$ 0.2	4.6 $\pm$ 0.6 a	16.0 $\pm$ 2.0
<i>Priestia</i> sp. U3	56.8 $\pm$ 4.1	2.0 $\pm$ 0.2	15.2 $\pm$ 1.2 a	1.1 $\pm$ 0.1	4.5 $\pm$ 0.4 a	18.0 $\pm$ 1.4 a
Bacterial consortium	50.4 $\pm$ 5	2.2 $\pm$ 0.2 a	15.5 $\pm$ 1.5 ab	1.5 $\pm$ 0.2	5 $\pm$ 0.5 ab	14 $\pm$ 1.3
<i>P</i> value	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001

Overall, the effects of fungicide application varied from complete inhibition to delayed bacterial growth. While FG only partly suppressed colony growth of isolates, PHFC was able to fully inhibit several bacterial isolates (Fig. 4). Both *Pantoea* isolates were resistant to the fungicides.

3.5 Influence of antimicrobial solutions on seed germination and endophytic microbiome of seedlings

Metrosideros excelsa seeds collected from three different trees began germinating after five days, and two days later, virtually all viable seeds germinated. The average number of emergent seedlings was 169.3 $\pm$ 18.2 from 120 mg of seeds

in the sterile water control and 167.7 $\pm$ 24.3 from the seeds treated with antimycotic antibiotic solution. The germination rate of seeds treated with antibiotic antimycotic solution for the in-vitro propagation did not significantly differ from water control (Welch's *t*-test, $P = 0.9588$, Table S4).

However, the early development of seedlings was impaired by antibiotics: three days after germination hypocotyls of treated seedlings were thinner, the radicles were elongating slower, and they did not develop root hairs as control seedlings (Fig. 5). One week after the germination, control seedlings developed long primary roots covered in dense root hairs, while treated seedlings only enlarged their lower hypocotyls. Despite initial treatment with antibiotic antimycotic solution, fungal growth was observed around

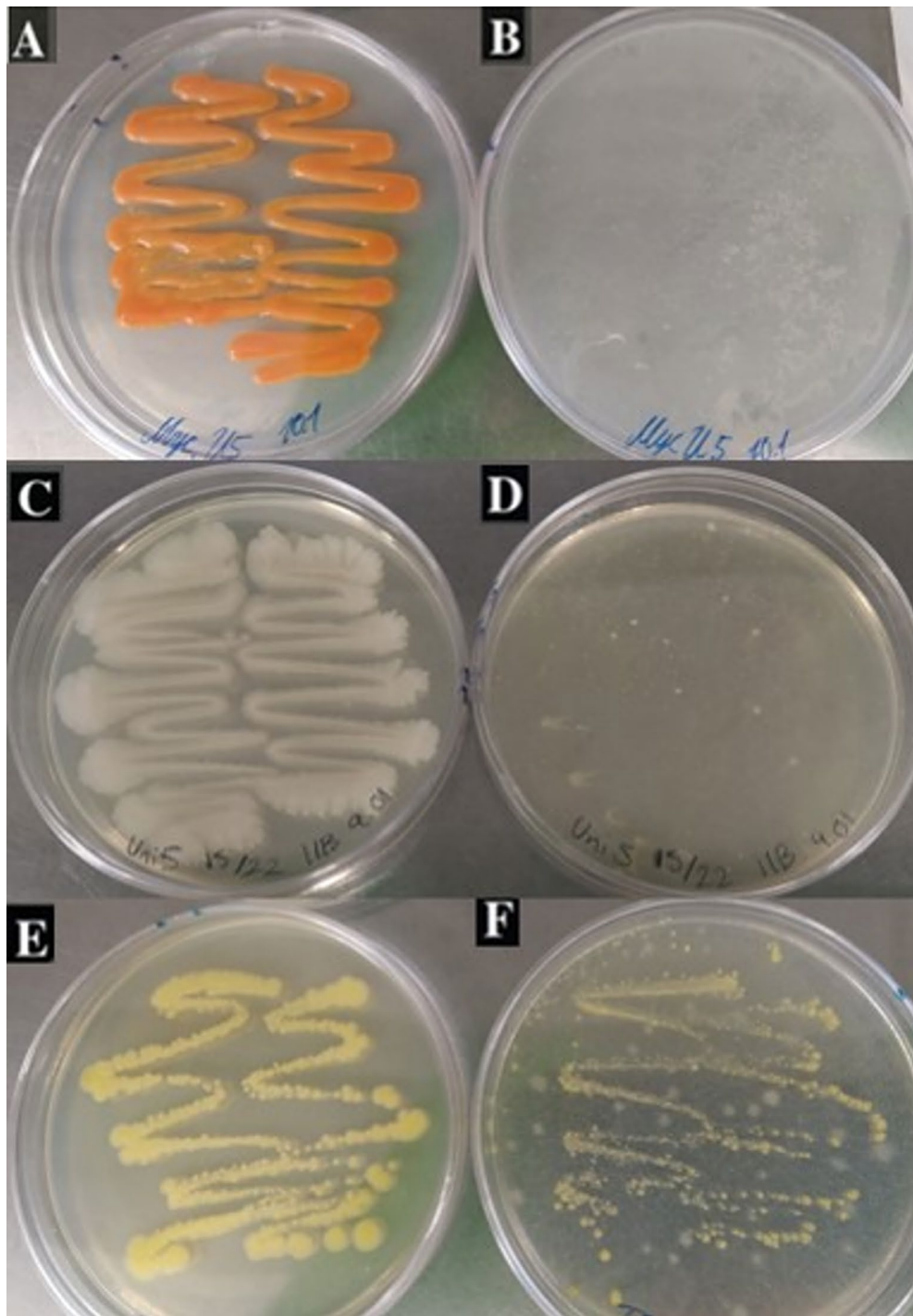


Fig. 4 Bacterial sensitivity to fungicides after one-week incubation. (A) *Mycolicibacterium* sp. U5 control plate. (B) Complete inhibition of *Mycolicibacterium* sp. U5 by ready-to-use mix of fungicides Plant Health Fungus Control (Kiwicare, New Zealand, boscalid 0.25 g/L, pyraclostrobin 0.13 g/L, and tebuconazole 0.43 g/L.). (C) *Bacillus* sp.

U5 control plate. (D) *Bacillus* sp. U5 inhibited by Plant Health Fungus Control. (E) *Kocuria* sp. TD1 control plate. (F) Growth of *Kocuria* sp. TD1 impaired by commercial fungicide Fungus Gun (Yates, Australia, 0.05 g/litre myclobutanil)

treated seedlings; therefore, additional treatment with systemic fungicide PHFC was applied together with antibiotic antimycotic solution to obtain endophyte-free seedlings.

Despite seed treatment with antibiotics, *Rahnella aquatilis* and *Pseudomonas* sp. were recovered from control and treated seedlings derived from Tree 1. *Pseudomonas* sp. was also present in Tree 2 control and treated seedlings. Tree



Fig. 5 Seedlings of *Metrosideros excelsa*. (A) Three days after germination, treated with antibiotic antimycotic solution (62.5 µg/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B in citrate buffer; Sigma Aldrich, USA), and (B) water control. One week

after germination– (C) treated with antibiotic antimycotic solution and (D) water control. Seedlings grown from untreated seeds developed long roots with dense root hairs. Scale bars = 1000 µm

3 control and treated seedlings contained *Burkholderia* sp., whereas only treated seedlings yielded *Rhodopseudomonas* sp. on media. Endophytic fungi belonging to genera *Cladosporium* and *Aureobasidium* were isolated from control seedlings from Trees 1 and 2 but not from the treated seedlings. Tree 1 and 3 control seedlings contained two unknown species of *Penicillium*; one of them was also isolated from two sections of leaves from the treated Tree 3 seedlings. No fungi were present in the roots of any other treated seedlings. During the isolation of *Penicillium* sp. W5, we observed that this fungus could cause callus formation and root growth from the leaves, which developed long roots covered in mycelium (Fig. 6). Seedling pieces derived from other trees also actively developed roots in the presence of this fungus; therefore, the growth-promoting ability

of *Penicillium* sp. W5 was tested further in the seed inoculation experiment.

3.6 Seed inoculation experiment

Seedlings derived from a single tree can develop different morphotypes when inoculated with different microbes (Fig. 7). Some seedlings grew as single stem shoots whereas others produced branches from the stem at the early stages of their development. Shoot apical meristems of seedlings inoculated with certain bacteria were sometimes divided into 2–4 equal stems (Fig. 7; Fig. S1). Growth parameters of seedling roots and shoots varied significantly between the treatments and control groups ($P \leq 0.0006$, Table 4, Tables S5). Shoots of the seedlings sterilised with antimicrobial solutions were significantly shorter than those of

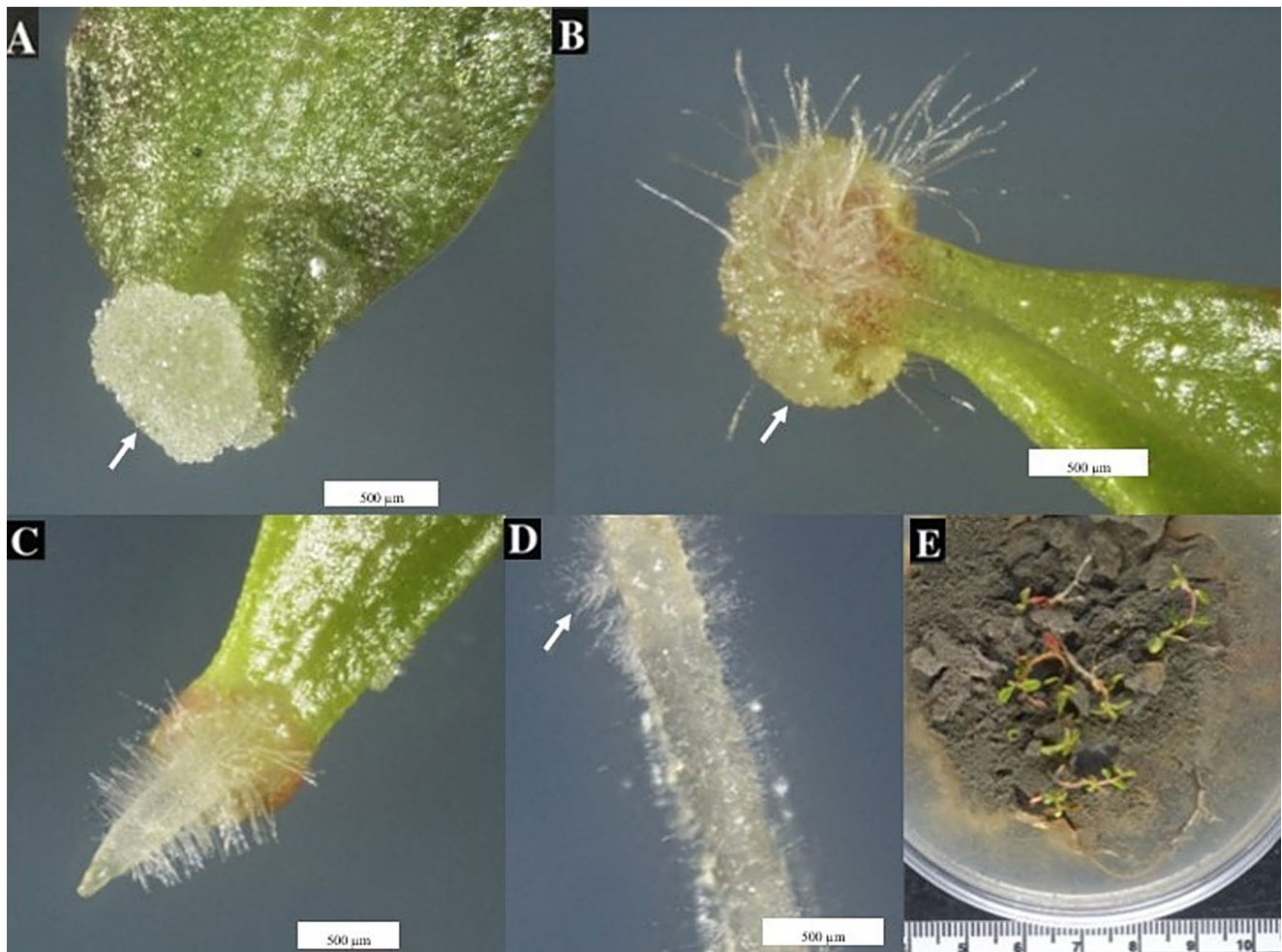


Fig. 6 *Penicillium* sp. W5 initiates callus formation from the leaves (**A**, **B**); arrows point at callus. Root formation (**C**) and mycelial growth (arrow) from the root (**D**); seedling cuttings develop roots in a plate containing the fungus (**E**). Scale bars for A–D = 500 µm

water control and all the treatments, except inoculation with *Bacillus* sp. U5, which was the only strain that did not promote seedling growth compared to the controls (Table 4, Tables S5). Root branching was another parameter significantly impaired after antimicrobial treatment ($P < 0.0001$, Tables S5). Seedlings from the sterilised control often produced only a taproot with small side roots whereas water control seedlings and plants inoculated with *Penicillium* sp. W5, *Bacillus* sp. W2, *Kocuria* sp. TD1, *Methylobacterium* sp. Tw3, *Pantoea* sp. TD1, *Paenibacillus* sp. Tw2, and the bacterial consortium developed dense fibrous root architectures (Fig. 7; Fig. S1).

Kocuria sp. TD1 was the only isolate that enhanced seedling growth across all measured parameters except root branching. The organs of inoculated seedlings significantly differed from those of sterilised control and water control with treated plants developing longer roots and larger and heavier shoots with more leaf pairs and side branches. The fresh weight for seedlings inoculated with *K. palustris* TD1

was four times (34.0 ± 3.8 mg) heavier than the sterilised control (8.0 ± 1.4 mg) and close to three times heavier than the water control (12.0 ± 1.0 mg), respectively. In addition, *Kocuria* sp. TD1 significantly increased seedling shoot weight compared to all other inoculated groups, i.e., *Penicillium* sp. W5, bacterial consortium, *Priestia* sp. U3, *Bacillus* sp. W2, *Pantoea* sp. TD1, *Bacillus* sp. U5, *Mycolicibacterium* sp. U5, *Methylobacterium* sp. Tw3, and *Pantoea* sp. OTH1 (Table 4; Tables S5).

Seedlings inoculated with *Penicillium* sp. W5 had more densely branching roots and higher shoots in comparison to the sterilised control and water control groups ($P < 0.0001$), but the number of leaf pairs (4.8 ± 0.4) and shoot fresh weight (19.5 ± 1.3 mg) significantly differed ($P = 0.0001$) only from sterilised control (2.9 ± 0.6 and 8.0 ± 1.4 , respectively). Treatment with *Paenibacillus* sp. Tw2 resulted in a similar seedling response (Table 4). In contrast, *Bacillus* sp. W2, and *Mycolicibacterium* sp. U5 significantly increased shoot weight in comparison to water and sterilised controls.

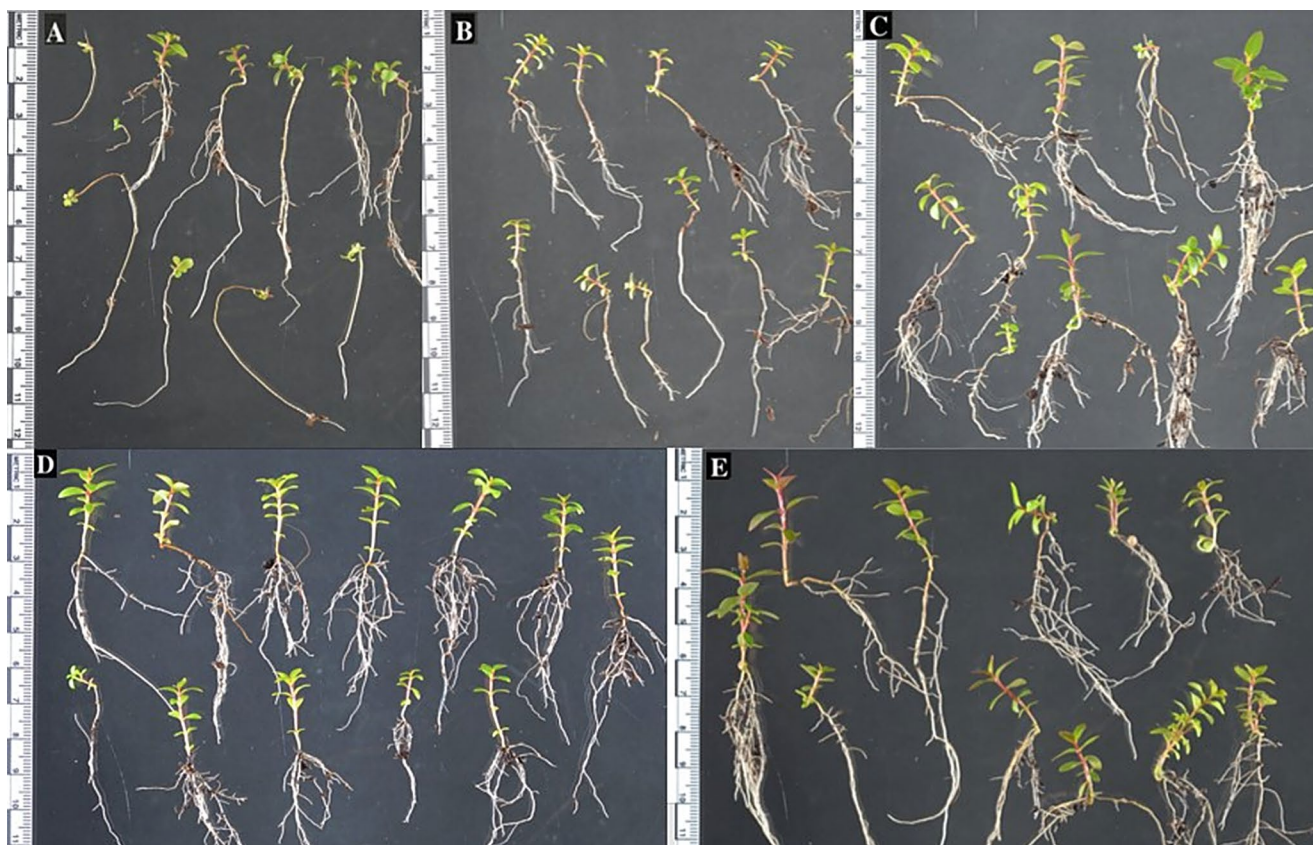


Fig. 7 Seedlings after seed inoculation experiment. Seeds were treated with antibiotic antimycotic solution and Plant Health Fungus Control, then inoculated with microbial isolates identified as beneficial for plant growth and/or protection against *A. psidii* in previous experiments. (A) Treated control— seeds were treated and propagated in sterile substrate in axenic conditions. (B) Water control. (C) Treated seeds were inocu-

lated with *Pantoea* sp. TD1; (D) inoculated with *Penicillium* sp. W5; and (E) inoculated with *Kocuria* sp. TD1. Inoculated seedlings demonstrate longer shoot internodes and more developed lateral roots than control seedlings. Some seedlings inoculated with *Pantoea* and *Kocuria* developed branches or two-three equal stems, whereas *Penicillium* did not induce branching

Inoculation with the bacterial consortium resulted in a broad variation of seedling morphotypes in each container; some seedlings developed 2–3 stems from apical meristem, others developed one stem with robust leaves, and root development also varied (Fig. S1). Another treatment that also demonstrated broad variation of seedling response was inoculation with *Bacillus* sp. U5, where the development of some seedlings stagnated while others demonstrated more active growth.

3.7 Recovery of inoculated taxa through a culture-based approach

Different species were isolated from the shoots and roots of seedlings (Table 5). All inoculated isolates were reisolated on media from the tissues of the seedlings, except *Kocuria* sp. TD1. Although this bacterium was present in soil, it was not recovered from the seedlings (Table 5). However, a few bacterial species were present not only in deliberately inoculated seedlings but also in seedlings inoculated with

other taxa. Thus, *Paenibacillus* sp. was isolated from three groups of seedlings initially inoculated with *Kocuria* sp. TD1, *Priestia* sp. U3, and *Penicillium* sp. W5, respectively. *Pantoea* spp. were recovered from seedlings inoculated with *Bacillus* sp. U5, and *Methylobacterium* sp. Tw3. *Aureobasidium* sp. was present in seedlings treated with *Kocuria* sp. TD1 and *Priestia* sp. U3. *Penicillium* sp. OTH4 was present in water control seedlings, sterilised control seedlings, and seedlings inoculated with *Paenibacillus* sp. Tw2, *Kocuria* sp. TD1, *Priestia* sp. U3, *Bacillus* sp. OTH5, and *Mycolicibacterium* sp. U5 (Table 5). Interestingly, *Pseudomonas* sp. was abundant only in seedlings from sterilised seeds, whereas seedlings from the water control group contained mostly *Cladosporium* aff. *halotolerans*, and *Microbacterium* sp.

Table 5 Taxa recovered from roots and shoots of seedlings three months after inoculation

Inoculated	Taxa isolated (closest GenBank match, number, percent identity)	
	Roots	Shoots
<i>Paenibacillus</i> sp. Tw2	<i>Bacillus subtilis</i> (OP658876, 100%)	<i>Paenibacillus</i> sp. G2Bc6 (KF465973, 99.86%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%)
<i>Kocuria</i> sp. TD1	<i>Priestia aryabhattai</i> (OQ547178, 99.91%) 99.71% <i>Paenibacillus</i> sp. G2Bc6 (KF465973.1, 99.71%)	<i>Paenibacillus</i> sp. G2Bc6 (KF465973, 99.86%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%); <i>Aureobasidium</i> sp. (OK376894, 100%)
<i>Priestia</i> sp. aff. <i>aryabhattai</i> U3	<i>Priestia aryabhattai</i> (MK519060.1, 99.78%) <i>Paenibacillus</i> sp. G2Bc6 16 S (KF465973, 99.85%)	<i>Priestia aryabhattai</i> (MK519060, 99.78%); <i>Paenibacillus</i> sp. G2Bc6 16 S (KF465973, 99.85%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%); <i>Aureobasidium</i> sp. (OK376894, 100%)
<i>Bacillus</i> sp. aff. <i>subtilis</i> OTH5	<i>Bacillus subtilis</i> (LC802086, 100%)	<i>Bacillus subtilis</i> (LC802086, 100%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%)
<i>Pantoea</i> sp. aff. <i>ananatis</i> TD1	<i>Pantoea ananatis</i> (MG574389.1, 100%)	<i>Pantoea ananatis</i> (MG574389.1, 100%);
<i>Bacillus</i> sp. U5	<i>Bacillus subtilis</i> (AY881642, 99.91%); <i>Pantoea ananatis</i> (CP054912, 99.76%)	<i>Bacillus subtilis</i> (AY881642, 99.91%); <i>Pantoea ananatis</i> (CP054912, 99.76%)
<i>Mycolicibacterium</i> sp. U5	<i>Priestia aryabhattai</i> or <i>megaterium</i> (CP146500, PP463907, 99.86%); <i>Mycolicibacterium</i> sp. U5 (OR472927, 100%)	<i>Priestia aryabhattai</i> or <i>megaterium</i> (CP146500, PP463907, 99.86%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%)
<i>Methylobacterium</i> sp. Tw3	<i>Pantoea</i> sp. (KY074223, 99.61%)	<i>Pantoea</i> sp. (KY074223, 99.63%) <i>Methylobacterium</i> sp. (PP716427, 100%)
<i>Pantoea</i> sp. OTH1	<i>Bacillus subtilis</i> (LC804382, 100%); <i>Pantoea agglomerans</i> (PP417696, 99.87%)	<i>Pantoea agglomerans</i> (PP417696, 99.87%)
Consortium of bacteria 1–9	<i>Bacillus subtilis</i> (AY881642, 99.91%); <i>Bacillus subtilis</i> (LC802086, 100%); <i>Bacillus subtilis</i> (MT229229, 99.91%) <i>Mycolicibacterium</i> sp. U5 (OR472927); <i>Priestia aryabhattai</i> (MK519060, 99.78%);	<i>Pantoea</i> sp. (MZ289970, 98.76%); <i>Bacillus subtilis</i> (MT229229, 99.91%) <i>Methylobacterium</i> sp. (PP716427, 100%)
<i>Penicillium</i> sp. W5	<i>Penicillium</i> sp. W5 (PP708937, 100%); <i>Paenibacillus</i> sp. G2Bc6 1 (KF465973, 99.86%)	<i>Penicillium</i> sp. W5 (PP708937, 100%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%); <i>Paenibacillus</i> sp. G2Bc6 1 (KF465973, 99.86%)
Sterilised control	<i>Pseudomonas</i> sp. (OL824686, 99.93%)	<i>Pseudomonas</i> sp. (OL824686, 99.93%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%)
Water control	<i>Cladosporium</i> aff. <i>halotolerans</i> (MN859971, 99.62%); <i>Microbacterium testaceum</i> (MK618600, 100%)	<i>Cladosporium</i> aff. <i>halotolerans</i> (MN859971, 99.62%); <i>Microbacterium testaceum</i> (MK618600, 100%); <i>Penicillium</i> sp. OTH4 (PP708938, 100%)

4 Discussion

We used microorganisms isolated from the seed coat and inner tissues of *Metrosideros excelsa* seeds aseptically extracted from still closed seed capsules. Previous studies indicated that bacteria isolated from seeds and foliage have been shown to suppress or promote the growth of fungi isolated from the same plant species (Christian et al. 2021; Newcombe et al. 2023). Our results demonstrate that seed-borne bacteria from *Bacillus*, *Peribacillus*, and *Priestia* are potent antagonists of most of the seed-associated fungi tested, including genera that contain species pathogenic to plants such as *Colletotrichum*, *Cytospora*, and *Neofusicoccum*, as well as putative beneficial endophytes such as *Aureobasidium* and *Cladosporium*. This implies that if strongly antagonistic bacteria enter developing seeds before fungi, these bacterial species may occupy the seeds and suppress fungi arriving later.

Some bacterial taxa are known to promote the growth of certain species of fungi and suppress others (del Barrio-Duque Alejandro et al. 2020; Christian et al. 2021;

Newcombe et al. 2023). Although recent work (del Barrio-Duque et al. 2019, 2020) has demonstrated that several bacterial isolates stimulate beneficial fungal endophytes, the results of our study are more similar to those observed by Christian et al. (2021). We found that three bacterial isolates promoted the growth of *Alternaria* and *Cladosporium* but inhibited the growth of *Cytospora*. *Alternaria* species are often pathogenic to plants, including Myrtaceae, and *Cytospora* species are associated with cankers and dieback of juvenile eucalypts (Adams et al. 2005; Lupo et al. 2001; Crous et al. 2019; Pan et al. 2020). However, certain *Alternaria* species may also be plant mutualists (DeMers 2022; Elghaffar et al. 2022). Consequently, if bacteria stimulate the growth of fungi beneficial for their plant host, such interactions might potentially have cumulative positive effects on plant growth and resilience to environmental stresses, whereas bacterial facilitation of phytopathogenic fungi will likely cause rapid decline of the host; however, this hypothesis should be further tested *in planta*.

Based on observations during 2021–23 (Leonard 2023, 2024), *M. excelsa* trees displayed a wide variation in

susceptibility to myrtle rust, with resistant trees located close to heavily infected ones. An investigation of seed-borne microbiome of *M. excelsa* indicated that individual trees contain different assemblies of bacteria and fungi, whose composition varied among closely located trees (Kholostiaikov et al. 2024). As some trees accumulated potentially beneficial endophytes in their seeds, and other trees had phytopathogens, the seed microbial assembly could potentially have different influences on seedling survival. We suggest that depending on the source tree, bacteria and fungi may promote seedling growth and their resistance to pathogens, such as *A. psidii*, or alternately, the seed microbiome may adversely affect the seedling survival.

We tested the influence of bacterial isolates on germinating urediniospores of *A. psidii*, and the inhibitory effects of fungicides on bacterial growth. *Bacillus* isolates, as in other studies with rust fungi, were found to be potent inhibitors of *A. psidii* (Manjula et al. 2004; Li et al. 2013; Dorighello et al. 2015; Kiani et al. 2021; Khan et al. 2023). However, we found that most of the bacteria that suppressed urediniospore germination and inhibited the growth of seed-borne pathogens were sensitive to the Plant Health Fungus Control (PHFC) fungicide formulation. We propose that fungicide application to prevent myrtle rust disease may potentially diminish the bacterial population that protects against pathogenic fungi.

The off-target effects of fungicides is a new area of research, and available data suggest that different formulations display various effects on endophytic bacteria, from complete inhibition and cell wall degradation to growth facilitation (Sharma et al. 2022; Andreolli et al. 2023; Luong et al. 2024). Based on recent data, endophytic Bacillota and Actinomycetota are more sensitive to boscalid and tebuconazole than Pseudomonadota (Andreolli et al. 2023). Our results confirmed this trend, as all isolates of Bacillota and Actinomycetota were sensitive to PHFC, except *Priestia* sp. U3, which was sensitive to myclobutanil. Myclobutanil belongs to triazole fungicides, primarily targeting ergosterol of fungal cell walls, but derivatives of triazoles potentially inhibit bacterial enzymes (Matin et al. 2022; Andreolli et al. 2023; Roman et al. 2023). One recent study found that propiconazole, another triazole fungicide, inhibited endophytic bacteria in vitro and in planta, resulting in the reduction of beneficial bacteria in the host, making plant more susceptible to bacterial speck disease (Yadav et al. 2022). In contrast, another study demonstrated that growth-promoting Pseudomonadota from genera *Citrobacter* sp., *Klebsiella* sp., and *Pseudomonas* sp. were able to use triazole fungicides as carbon sources (Luong et al. 2024). Such selective sensitivity of bacteria to fungicides may explain why we isolated different microbial taxa from *M. excelsa* seedlings after the application of antimycotic antibiotic solution

for tissue culture and PHFC fungicide. *Cladosporium* and *Microbacterium* were potentially eliminated from seeds after treatment with antimicrobial solutions, and the subsequent application of fungicides could promote *Pseudomonas* proliferation in seedlings. The absence of *Pseudomonas* sp. in seedlings deliberately inoculated with other bacteria may be explained by their greater cell numbers in inoculum, enabling fast occupation of seeds. Since we placed germinating seeds in bacterial inoculum consisting of actively multiplying cells, these bacteria may have rapidly disseminated within plant tissues, outcompeting resident bacteria, which may have led to the ability to reisolate inoculated bacteria from tissues of seedlings after their three-month growth in closed containers, except for *Kocuria* sp. TD1.

However, seed treatment with *Kocuria* sp. TD1 most significantly enhanced seedling development compared to other inoculations and controls. Species of *Kocuria* are known as soil-dwelling growth-promoting bacteria, which can migrate to seeds and be vertically transmitted to the next generation (Sánchez-López et al. 2017; Liang et al. 2021; Sharma et al. 2023; Mauceri et al. 2024). This bacterium can support plant growth via the production of plant hormones, phosphate solubilisation, and resistance to salt, drought, and heavy metal pollution (Zacaria Vital et al. 2019; Wang et al. 2020; Afridi et al. 2021; Mauceri et al. 2024). Our results support these reports because seedlings inoculated with *Kocuria* sp. TD1 had the most developed root system and larger, heavier shoots with more branches than any other seedlings. Our failure to reisolate this bacterium from seedlings might result from the prolonged sterilisation of plant tissues, which may have killed the bacterium, or that *Kocuria* sp. was outcompeted by faster-growing bacteria on media. In the future, it may be necessary to use selective media for Actinomycetes or direct sequencing from plant material, as abundance of this genus can be relatively low in samples (Sánchez-López et al. 2017).

Unexpectedly, we isolated *Aureobasidium* sp., *Penicillium* sp. OTH4, *Paenibacillus* sp., and *Priestia* sp. from seedlings that were treated with antibiotics and fungicides and later inoculated with other microorganisms. These microbial taxa could be present in intact seeds and survive treatment, and thus, microorganisms inoculated later were incorporated into the existing microbiome. Endophytic *Aureobasidium* and some bacteria have been documented to exist intracellularly in seedlings (White et al. 2019). We suggest that such microorganisms may survive inside plant cells away from extracellular antimicrobial agents, or their concentration was insufficient to eliminate bacteria and fungi from the seeds. Potentially, only high antibiotic concentrations can eliminate bacteria from seeds (Puente et al. 2009).

In our experiments, antimicrobial solutions did not affect the germination of *M. excelsa* seeds, but treated seedlings had impaired root development. The same trend was observed for other plants (Minden et al. 2017; Bellino et al. 2018). It was also documented that seed-borne bacteria and fungi accumulate in roots, triggering the formation of dense root hairs, but treatment with antibiotics and fungicides significantly reduced endophytic diversity and root development (Puente et al. 2009; Irizarry and White 2017; Vasanthakumari et al. 2018).

Fungicides have been suggested as effective strategy for myrtle rust control in New Zealand, and antimycotic antibiotic solutions are used for plant tissue culture to save endangered Myrtaceae (Adusei-Fosu et al. 2021; van der Walt et al. 2023). Fungicide application to control myrtle rust on *Lophomyrtus* sp., New Zealand Myrtaceae, demonstrated that treated plants develop shorter internodes, but there is no explanation for this effect (Beresford and Wright 2022). In our experiments, shoots of *M. excelsa* seedlings after antimicrobial treatment were shorter than those of the water control. In contrast, when treated seeds were subsequently inoculated with seed-borne endophytes, the resulted seedlings developed longer internodes, larger leaves, and dense root systems. Thus, our results suggest that applying beneficial endophytes after antimicrobial treatment may stimulate seedling growth and introduce natural protection against pathogens. However, microorganisms antagonistic towards pathogens do not necessarily benefit plant growth, as demonstrated by our results with *Bacillus* sp. U5, which, despite being the most potent antagonist of *A. psidii* *in vitro*, did not improve the growth of inoculated seedlings.

During our experiments, we found that leaf cuttings of *M. excelsa* could produce roots. There is no evidence in literature about such root formation by Myrtaceae; however, we observed this phenomenon for seedling leaves that harboured *Penicillium* sp. W5. Dissected seedlings readily developed new roots in the presence of *Penicillium* sp. W5, and further seed inoculation experiments confirmed that this fungus was responsible for root branching. Some species of *Penicillium* may facilitate plant growth via phosphorous acquisition from soil, production of plant hormones and siderophores, and alleviation of salt stress (Wakelin et al. 2004, 2007; Babu et al. 2015; Molina-Montenegro et al. 2020). In addition, it was shown that *Penicillium simplicissimum* could induce resistance to *Puccinia striiformis* f. sp. *tritici* (Esmail et al. 2022).

We observed that *Penicillium* sp. co-occurred with *Paenibacillus* sp. in seedlings, and this bacterium was also isolated from presumably clean colonies of *Penicillium* sp. W5, which was responsible for callus and root formation from detached leaves of *M. excelsa* seedlings. The root formation may have resulted from the cumulative effects of

Penicillium and *Paenibacillus*, which occupied seedling tissues together. Metabolites of *Penicillium* species have been shown to activate the swarming motility of *Paenibacillus* sp. towards the mycelium (Park et al. 2008). *Paenibacillus* species are well-known growth-promoting bacteria often isolated from seeds (Ferreira et al. 2008; Rybakova et al. 2016; Liu et al. 2019; Singh and Wesemael 2022). These bacteria are common in tissue cultures of woody plants, initiating root formation from microcuttings (Ulrich et al. 2008).

In conclusion, our study demonstrated the importance of seed-borne bacteria and *Penicillium* isolates in developing *M. excelsa* seedlings. The results indicate the potential of several isolates as plant growth-promoting endophytes, which may be employed for sustainable plant nursery practices. We also demonstrated that it is impossible to eliminate all bacteria and fungi from seeds using the antibiotic antimycotic solution in recommended concentrations. Antibiotics and fungicides adversely affect seedling development; nevertheless, certain beneficial seed-borne isolates may be effectively inoculated into treated seeds. We suggest that inoculating seedlings with a combination of growth-promoting isolates and antagonists of *A. psidii* may provide seedlings with the best chance of survival.

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Author contributions VK conceived the project. VK and BB collected samples. VK conducted experiments and analysed the data with the support of BB, HR, and MP. VK drafted the manuscript, with all authors editing and approving the final text.

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Declarations

Conflict of interest The authors declare there is no scientific or economic conflict of interest between them or with any third person.

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References

- Adams G, Wingfield MJ, Common R, Roux J (2005) Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (Ascomycota, diarthales, Valsaceae) from *Eucalyptus*. *Stud Mycol* 52:1–142
- Adusei-Fosu K, Rolando CA, Richardson B, van Leeuwen R, Gasikin R, Bader MKF, Pathan AK (2021) Evaluating the efficacy of potential fungicide-adjuvant combinations for control of myrtle rust in New Zealand. *J Plant Dis Prot* 128(6):1501–1515. <https://doi.org/10.1007/s41348-021-00523-1>
- Afridi MS, Van Hamme Jd, Bundschuh J, Sumaira, Khan MN, Salam A, Waqar M, Munis MFH, Chaudhary HJ (2021) Biotechnological approaches in agriculture and environmental management - bacterium *Kocuria rhizophila* 14ASP as heavy metal and salt-tolerant plant growth-promoting strain. *Biologia* 76(10):3091–3105. <https://doi.org/10.1007/s11756-021-00826-6>
- Alvarado R, Sagredo-Saez C, Fuentes-Quiroz A, Villanueva-Guerrero A, Mujica MI, Ahumada R, Almonacid-Muñoz L, Jorquera-Fontena E, de Oliveira Costa PH, da Silva Valadares RB et al (2024) Endophytic seed-associated microbial diversity and its impact on seedling growth of the Andean tree *Nothofagus obliqua* (Mirb.) Oerst. *Plant Growth Regul*. <https://doi.org/10.1007/s10725-023-01113-x>
- Andreolli M, Lampis S, Tosi L, Marano V, Zapparoli G (2023) Fungicide sensitivity of grapevine bacteria with plant growth-promoting traits and antagonistic activity as non-target microorganisms. *World J Microbiol Biotechnol* 39(5):121. <https://doi.org/10.1007/s11274-023-03569-5>
- Ardanov P, Sessitsch A, Haggman H, Kozyrovska N, Pirttilä AM (2012) *Methylobacterium*-induced endophyte community changes correspond with protection of plants against pathogen attack. *PLoS ONE* 7(10):e46802. <https://doi.org/10.1371/journal.pone.0046802>
- Avenot HF, Michailides TJ (2010) Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot* 29(7):643–651. <https://doi.org/10.1016/j.cropro.2010.02.019>
- Babu AG, Kim SW, Yadav DR, Hyum U, Adhikari M, Lee YS (2015) *Penicillium menonorum*: A novel fungus to promote growth and nutrient management in cucumber plants. *Mycobiology* 43(1):49–56. <https://doi.org/10.5941/MYCO.2015.43.1.49>
- Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ (2002) Characterisation of systemic resistance in sugar beet elicited by a non-pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. *Physiol Mol Plant Pathol* 61(5):289–298. <https://doi.org/10.1006/pmpp.2003.0443>
- Bellino A, Lofrano G, Carotenuto M, Libralato G, Baldantoni D (2018) Antibiotic effects on seed germination and root development of tomato (*Solanum lycopersicum* L.). *Ecotoxicol Environ Saf* 148:135–141. <https://doi.org/10.1016/j.ecoenv.2017.10.006>
- Beresford RM, Wright PJ (2022) Risk-based fungicide management for myrtle rust in nurseries. Plant & Food Research report for Ministry for Primary Industries. July 2022. PFR SPTS No. 22715. <https://www.mpi.govt.nz/dmsdocument/54247-Risk-based-fungicide-management-for-myrtle-rust-in-nurseries>
- Bhattacharya A, Giri VP, Singh SP, Pandey S, Chauhan P, Soni SK, Srivastava S, Singh PC, Mishra A (2019) Intervention of bio-protective endophyte *Bacillus tequilensis* enhance physiological strength of tomato during *Fusarium* wilt infection. *Biol Control* 139. <https://doi.org/10.1016/j.biocontrol.2019.104074>
- Bokhari A, Essack M, Lafi FF, Andres-Barrao C, Jalal R, Alamoudi S, Razali R, Alzubaidy H, Shah KH, Siddique S et al (2019) Bioprospecting desert plant *Bacillus* endophytic strains for their potential to enhance plant stress tolerance. *Sci Rep* 9(1):18154. <https://doi.org/10.1038/s41598-019-54685-y>
- Bragard C, Baptista P, Chatzivassiliou E, Di Serio F, Gonthier P, Jaques Miret JA, Justesen AF, MacLeod A, Magnusson CS et al (2023) Pest categorisation of *Pantoea ananatis*. *EFSA J* 21(3):e07849
- Bylsma RJ, Clarkson BD, Efford JT (2014) Biological flora of New Zealand 14: *Metrosideros excelsa*, pōhutukawa, New Zealand Christmas tree. *N Z J Bot* 52(3):365–385. <https://doi.org/10.1080/0028825x.2014.926278>
- Chee-Sanford JC, Williams MM, Davis AS, Sims GK (2006) Do microorganisms influence seed-bank dynamics? *Weed Sci* 54(3):575–587. <https://doi.org/10.1614/WS-05-055R.1>
- Christian N, Espino Basurto B, Toussaint A, Xu X, Ainsworth EA, Busby PE, Heath KD (2021) Elevated carbon dioxide reduces a common soybean leaf endophyte. *Glob Chang Biol* 27(17):4154–4168. <https://doi.org/10.1111/gcb.15716>
- Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, Summerell BA, Edwards J, Taylor PWJ, Groenewald JZ (2019) Foliar pathogens of eucalypts. *Stud Mycol* 94:125–298. <https://doi.org/10.1016/j.simyco.2019.08.001>
- da Silva NMP, Guterres DC, Borges LS, Barreto RW, Furtado GQ (2023) Surveying potentially antagonistic fungi to myrtle rust (*Austropuccinia psidii*) in Brazil: fungicolous *Cladosporium* spp. *Braz J Microbiol* 54(3):1899–1914. <https://doi.org/10.1007/s42770-023-01047-6>
- del Barrio-Duque A, Ley J, Samad A, Antonielli L, Sessitsch A, Compant S (2019) Beneficial endophytic bacteria-*Serendipita indica* interaction for crop enhancement and resistance to phytopathogens. *Front Microbiol* 10:2888. <https://doi.org/10.3389/fmicb.2019.02888>
- del Barrio-Duque A, Samad A, Nybroe O, Antonielli L, Sessitsch A, Compant S (2020) Interaction between endophytic Proteobacteria strains and *Serendipita indica* enhances biocontrol activity against fungal pathogens. *Plant Soil* 451(1–2):277–305. <https://doi.org/10.1007/s11104-020-04512-5>
- DeMers M (2022) *Alternaria alternata* as endophyte and pathogen. *Microbiology* 168(3). <https://doi.org/10.1099/mic.0.001153>
- Deng C, Liang X, Zhang N, Li B, Wang X, Zeng N (2022) Molecular mechanisms of plant growth promotion for methylotrophic *Bacillus aryabhattai* LAD. *Front Microbiol* 13:917382. <https://doi.org/10.3389/fmicb.2022.917382>
- Di Francesco A, Di Foggia M, Corbetta M, Baldo D, Ratti C, Baraldi E (2020) Biocontrol activity and plant growth promotion exerted by *Aureobasidium pullulans* strains. *J Plant Growth Regul* 40(3):1233–1244. <https://doi.org/10.1007/s00344-020-10184-3>
- Doilom M, Guo JW, Phookamsak R, Mortimer PE, Karunarathna SC, Dong W, Liao CF, Yan K, Pem D, Suwannarach N et al (2020) Screening of phosphate-solubilising Fungi from air and soil in Yunnan, China: four novel species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. *Front Microbiol* 11:585215. <https://doi.org/10.3389/fmicb.2020.585215>

- Dorighello DV, Bettiol W, Maia NB, de Campos Leite RMVB (2015) Controlling Asian soybean rust (*Phakopsora pachyrhizi*) with *Bacillus* spp. and coffee oil. *Crop Prot* 67:59–65. <https://doi.org/10.1016/j.cropro.2014.09.017>
- Eevers N, Gielen M, Sanchez-Lopez A, Jaspers S, White JC, Vangronsveld J, Weyens N (2015) Optimization of isolation and cultivation of bacterial endophytes through addition of plant extract to nutrient media. *Microb Biotechnol* 8(4):707–715. <https://doi.org/10.1111/1751-7915.12291>
- Egamberdieva D, Alimov J, Shurigin V, Alaylar B, Wirth S, Bellin-grath-Kimura SD (2021) Diversity and plant growth-promoting ability of endophytic, halotolerant bacteria associated with *Tetragonia tetragonioides* (Pall.) Kuntze. *Plants* 11(1). <https://doi.org/10.3390/plants11010049>
- Elghaffar RYA, Amin BH, Hashem AH, Sehim AE (2022) Promising endophytic *Alternaria alternata* from leaves of *Ziziphus spinachristi*: phytochemical analyses, antimicrobial and antioxidant activities. *Appl Biochem Biotechnol* 194(9):3984–4001. <https://doi.org/10.1007/s12010-022-03959-9>
- Esmail SM, Draz IS, Saleem MH, Mumtaz S, Elsharkawy MM (2022) *Penicillium simplicissimum* and *Trichoderma asperellum* counteract the challenge of *Puccinia striiformis* f. Sp. *tritici* in wheat plants. *Egypt J Biol Pest Control* 32(1). <https://doi.org/10.1186/s41938-022-00614-7>
- Ferreira A, Quecine MC, Lacava PT, Oda S, Azevedo JL, Araujo WL (2008) Diversity of endophytic bacteria from *Eucalyptus* species seeds and colonization of seedlings by *Pantoea agglomerans*. *FEMS Microbiol Lett* 287(1):8–14. <https://doi.org/10.1111/j.1574-6968.2008.01258.x>
- Fort T, Pauvert C, Zanne AE, Ovaskainen O, Caignard T, Barret M, Compant S, Hampe A, Delzon S, Vacher C (2021) Maternal effects shape the seed microbiome in *Quercus petraea*. *New Phytol* 230(4):1594–1608. <https://doi.org/10.1111/nph.17153>
- Franić I, Eschen R, Allan E, Hartmann M, Schneider S, Prospero S (2020) Drivers of richness and community composition of fungal endophytes of tree seeds. *FEMS Microbiol Ecol* 96(9). <https://doi.org/10.1093/femsec/fiaa166>
- Fulkerson RS, Tossell WE (1955) Seed treatment of forage legumes and grasses with three antibiotics. *Can J Agricultural Sci* 35(3):259–263. <https://doi.org/10.4141/agsci-1955-0035>
- Goggin DE, Emery RJ, Kurepin LV, Powles SB (2015) A potential role for endogenous microflora in dormancy release, cytokinin metabolism and the response to Fluridone in *Lolium rigidum* seeds. *Ann Bot* 115(2):293–301. <https://doi.org/10.1093/aob/mcu231>
- González AJ, Estefanía T (2020) Strains of *Neopestalotiopsis* sp. are in vitro antagonists of *Cryphonectria parasitica*. *Biol Control* 143. <https://doi.org/10.1016/j.biocontrol.2020.104187>
- Hanin NA, Fitriarsi PD (2019) Identification of Endophytic Fungi from Fruits and Seeds of Jambolana (*Syzygium cumini* L.) Skeels. *IOP conference series Earth and environmental science* 276(1):12060. <https://doi.org/10.1088/1755-1315/276/1/012060>
- Hashem A, Tabassum B, Fathi Abd Allah E (2019) *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi J Biol Sci* 26(6):1291–1297. <https://doi.org/10.1016/j.sjbs.2019.05.004>
- Hillis DG, Fletcher J, Solomon KR, Sibley PK (2011) Effects of ten antibiotics on seed germination and root elongation in three plant species. *Arch Environ Contam Toxicol* 60(2):220–232. <https://doi.org/10.1007/s00244-010-9624-0>
- Irizarry I, White JF (2017) Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. *J Appl Microbiol* 122(4):1110–1120. <https://doi.org/10.1111/jam.13414>
- Ismail I, Hamayun M, Hussain A, Iqbal A, Khan S, Gul S, Khan H, Rehman K, Bibi H, Lee I-J (2021) *Penicillium glabrum* acted as a heat stress relieving endophyte in soybean and sunflower. *Pol J Environ Stud* 30(4):3099–3110. <https://doi.org/10.15244/pjoes/128579>
- Jeong S, Kim TM, Choi B, Kim Y, Kim E (2021) Invasive *Lactuca serriola* seeds contain endophytic bacteria that contribute to drought tolerance. *Sci Rep* 11(1):13307. <https://doi.org/10.1038/s41598-021-92706-x>
- Karadimos DA, Karaoglanidis GS, Tzavella-Klonari K (2005) Biological activity and physical modes of action of the Qo inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*. *Crop Prot* 24(1):23–29. <https://doi.org/10.1016/j.cropro.2004.06.004>
- Khan MA, Raheel M, Khan SA, Abid AD, Shahzad S, Siddiqui HZ, Atif M, Hanif A (2023) Eco-friendly management of wheat stripe rust through application of *Bacillus subtilis* in combination with plant defense activators. *J King Saud Univ - Sci* 35(4):102587. <https://doi.org/10.1016/j.jksus.2023.102587>
- Kholostiaikov V, Burns B, Ridgway H, Padamsee M (2024) Variation in seed-borne microbial communities of *Metrosideros excelsa* sol. Ex Gaertn. with consequences for germination success. *New Z J Bot* 1–24. <https://doi.org/10.1080/0028825x.2024.2331573>
- Kiani T, Mehboob F, Hyder MZ, Zainy Z, Xu L, Huang L, Farakh S (2021) Control of stripe rust of wheat using indigenous endophytic bacteria at seedling and adult plant stage. *Sci Rep* 11(1):14473. <https://doi.org/10.1038/s41598-021-93939-6>
- Kohl J, Scheer C, Holb IJ, Masny S, Molhoek W (2015) Toward an integrated use of biological control by *Cladosporium cladosporioides* H39 in Apple scab (*Venturia inaequalis*) management. *Plant Dis* 99(4):535–543. <https://doi.org/10.1094/PDIS-08-14-0836-RE>
- Kurniawan A, Chuang H (2022) Rhizobacterial *Bacillus mycoides* functions in stimulating the antioxidant defence system and multiple phytohormone signalling pathways to regulate plant growth and stress tolerance. *J Appl Microbiol* 132(2):1260–1274. <https://doi.org/10.1111/jam.15252>
- Leonard J (2023) Concern for pōhutukawa grows following widespread myrtle rust infection. *Beyond Myrtle Rust*. <https://www.landcareresearch.co.nz/discover-our-research/biodiversity-biosecurity/ecosystem-resilience/beyond-myrtle-rust/news/concern-for-pohutukawa-grows-following-widespread-myrtle-rust-infection/>
- Leonard J (2024) Summer students: gaining experience while contributing new knowledge. *Beyond Myrtle Rust*. <https://www.landcareresearch.co.nz/discover-our-research/biodiversity-biosecurity/ecosystem-resilience/beyond-myrtle-rust/news/summer-students-gaining-experience-while-contributing-new-knowledge/>
- Li H, Zhao J, Feng H, Huang L, Kang Z (2013) Biological control of wheat stripe rust by an endophytic *Bacillus subtilis* strain E1R-j in greenhouse and field trials. *Crop Prot* 43:201–206. <https://doi.org/10.1016/j.cropro.2012.09.008>
- Li L, Gao L, Liu Y, Fang B, Huang Y, Mohamad OAA, Egamberdieva D, Li W, Ma J (2021) Diversity of cultivable endophytic bacteria associated with halophytes in Xinjiang of China and their plant beneficial traits. *J Arid Land* 13(8):790–800. <https://doi.org/10.1007/s40333-021-0016-2>
- Liang D, Guo J, Hou F, Bowatte S (2021) High level of conservation and diversity among the endophytic seed bacteriome in eight alpine grassland species growing at the Qinghai Tibetan plateau. *FEMS Microbiol Ecol* 97(6). <https://doi.org/10.1093/femsec/fiab060>
- Liu X, Li Q, Li Y, Guan G, Chen S (2019) *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. *PeerJ* 7:e7445. <https://doi.org/10.7717/peerj.7445>
- Liu T-T, Gou L-J, Zeng H, Zhou G, Dong W-R, Cui Y, Cai Q, Chen Y-X (2022) Inhibitory effect and mechanism of dill seed essential oil on *Neofusicoccum parvum* in Chinese chestnut. *Separations* 9(10). <https://doi.org/10.3390/separations9100296>

- Lu L, Chang M, Han X, Wang Q, Wang J, Yang H, Guan Q, Dai S (2021) Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. *J Appl Microbiol* 131(4):1919–1931. <https://doi.org/10.1111/jam.15082>
- Luo L, Zhao C, Wang E, Raza A, Yin C (2022) *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: an overview for its mechanisms. *Microbiol Res* 259:127016. <https://doi.org/10.1016/j.micres.2022.127016>
- Luong TT, Nguyen THT, Nguyen TD, Le VT, Pham THT, Ho TT, Nguyen NL (2024) Degradation of triazole fungicides by plant growth-promoting bacteria from contaminated agricultural soil. *J Microbiol Biotechnol* 34(1):56–64. <https://doi.org/10.4014/jmb.2308.08037>
- Lupo S, Tiscornia S, Bettucci L (2001) Endophytic fungi from flowers, capsules and seeds of *Eucalyptus globulus*. *Rev Iberoam Micol* 18(1):38–41
- Madhaiyan M, Alex TH, Ngoh ST, Prithiviraj B, Ji L (2015) Leaf-residing *Methylobacterium* species fix nitrogen and promote biomass and seed production in *Jatropha curcas*. *Biotechnol Biofuels* 8:222. <https://doi.org/10.1186/s13068-015-0404-y>
- Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014) *Pestalotiopsis* revisited. *Stud Mycol* 79:121–186. <http://doi.org/10.1016/j.simyco.2014.09.005>
- Mancini V, Romanazzi G (2014) Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest Manag Sci* 70(6):860–868. <https://doi.org/10.1002/ps.3693>
- Mangwende E, Chirwa PW, Aveling TAS (2020a) Evaluation of seed treatments against *Colletotrichum kahawae* subsp. *cigarro* on *Eucalyptus* spp. *Crop Protection* 132:105113. <https://doi.org/10.1016/j.cropro.2020.105113>
- Mangwende E, Chirwa PW, Aveling TAS (2020b) Seed health status and germination of *Eucalyptus* spp. *Eur J Plant Pathol* 159(1):55–65. <https://doi.org/10.1007/s10658-020-02140-4>
- Manjula K, Kishore GK, Podile AR (2004) Whole cells of *Bacillus subtilis* AF 1 proved more effective than cell-free and chitinase-based formulations in biological control of citrus fruit rot and groundnut rust. *Can J Microbiol* 50(9):737–744. <https://doi.org/10.1139/w04-058>
- Martin PL, Peter KA (2021) Quantification of *Colletotrichum fioriniae* in orchards and deciduous forests indicates it is primarily a leaf endophyte. *Phytopathology* 111(2):333–344. <https://doi.org/10.1094/PHYTO-05-20-0157-R>
- Matin MM, Matin P, Rahman MR, Ben Hadda T, Almalki FA, Mahmud S, Ghoneim MM, Alruwaily M, Alshehri S (2022) Triazoles and their derivatives: chemistry, synthesis, and therapeutic applications. *Front Mol Biosci* 9:864286. <https://doi.org/10.3389/fmolb.2022.864286>
- Mauceri A, Puccio G, Faddetta T, Abbate L, Polito G, Caldiero C, Renzone G, Lo Pinto M, Alibrandi P, Vaccaro E et al (2024) Integrated omics approach reveals the molecular pathways activated in tomato by *Kocuria rhizophila*, a soil plant growth-promoting bacterium. *Plant Physiol Biochem* 210:108609. <https://doi.org/10.1016/j.plaphy.2024.108609>
- Meena, Tara N, Saharan BS (2017) Plant growth promoting traits shown by bacteria *Brevibacterium frigiditolerans* SMA23 isolated from *Aloe vera* rhizosphere. *Agricultural Sci Digest– Res J* 37:226–231. <https://doi.org/10.18805/asd.v37i03.9223>
- Minden V, Deloy A, Volkert AM, Leonhardt SD, Pufal G (2017) Antibiotics impact plant traits, even at small concentrations. *AoB Plants* 9(2):plx010. <https://doi.org/10.1093/aobpla/plx010>
- Molina-Montenegro MA, Acuna-Rodriguez IS, Torres-Diaz C, Gundel PE, Dreyer I (2020) Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na(+) sequestration. *Sci Rep* 10(1):5819. <https://doi.org/10.1038/s41598-020-62544-4>
- Montealegre JR, Reyes R, Pérez LM, Herrera R, Silva P, Besoain X (2003) Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia Solani* in tomato. *Electron J Biotechnol* 6(2). <https://doi.org/10.2225/vol6-issue2-fulltext-8>
- Moumni M, Brodal G, Romanazzi G (2023) Recent innovative seed treatment methods in the management of seedborne pathogens. *Food Secur* 15(5):1365–1382. <https://doi.org/10.1007/s12571-023-01384-2>
- Murphy BR, Doohan FM, Hodkinson TR (2016) A seed dressing combining fungal endophyte spores and fungicides improves seedling survival and early growth in barley and oat. *Symbiosis* 71(1):69–76. <https://doi.org/10.1007/s13199-016-0418-7>
- Nelson EB (2017) The seed microbiome: origins, interactions, and impacts. *Plant Soil* 422(1–2):7–34. <https://doi.org/10.1007/s11104-017-3289-7>
- Newcombe G, Marlin M, Barge E, Heitmann S, Ridout M, Busby PE (2023) Plant seeds commonly host *Bacillus* spp., potential antagonists of phytopathogens. *Microb Ecol* 85(4):1356–1366. <https://doi.org/10.1007/s00248-022-02024-6>
- Pan M, Zhu H, Bonthond G, Tian C, Fan X (2020) High diversity of *Cytospora* associated with canker and dieback of Rosaceae in China, with 10 new species described. *Front Plant Sci* 11:690. <http://doi.org/10.3389/fpls.2020.00690>
- Park SY, Kim R, Ryu CM, Choi SK, Lee CH, Kim JG, Park SH (2008) Citrinin, a Mycotoxin from *Penicillium citrinum*, plays a role in inducing motility of *Paenibacillus polymyxa*. *FEMS Microbiol Ecol* 65(2):229–237. <https://doi.org/10.1111/j.1574-6941.2008.00492.x>
- Park YG, Mun BG, Kang SM, Hussain A, Shahzad R, Seo CW, Kim AY, Lee SU, Oh KY, Lee DY et al (2017) *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. *PLoS ONE* 12(3):e0173203
- Peres NA, Timmer LW, Adaskaveg JE, Correll JC (2005) Lifestyles of *Colletotrichum acutatum*. *Plant Dis* 89(8):784–796. <https://doi.org/10.1094/pd-89-0784>
- Prasannath K, Shivas RG, Galea VJ, Akinsanmi OA (2021) *Neopestalotiopsis* species associated with flower diseases of *Macadamia integrifolia* in Australia. *J Fungi* 7(9). <https://doi.org/10.3390/jof7090771>
- Puente ME, Li CY, Bashan Y (2009) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environ Exp Bot* 66(3):402–408. <https://doi.org/10.1016/j.envexpbot.2009.04.007>
- Qi D, Zou L, Zhou D, Zhang M, Wei Y, Zhang L, Xie J, Wang W (2021) Identification and antifungal mechanism of a nov elactinobacterium *Streptomyces huiliensis* Sp. Nov. Against *Fusarium oxysporum* f. Sp. *cubense* tropical race 4 of banana. *Front Microbiol* 12:722661. <https://doi.org/10.3389/fmicb.2021.722661>
- Qin Y, Pan X, Yuan Z (2016) Seed endophytic microbiota in a coastal plant and phytobeneficial properties of the fungus *Cladosporium cladosporioides*. *Fungal Ecol* 24:53–60. <https://doi.org/10.1016/j.funeco.2016.08.011>
- Reddy MS, Murali TS, Suryanarayanan TS, Govinda Rajulu MB, Thirunavukkarasu N (2016) *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of Southern India. *Fungal Ecol* 24:70–75. <https://doi.org/10.1016/j.funeco.2016.09.002>
- Roman DL, Matica MA, Ciorsac A, Boros BV, Isvoran A (2023) The effects of the fungicide myclobutanil on soil enzyme activity. *Agriculture* 13(10). <https://doi.org/10.3390/agriculture13101956>
- Rybakova D, Cernava T, Köberl M, Liebminger S, Etemadi M, Berg G (2016) Endophytes-assisted biocontrol: novel insights in ecology and the mode of action of *Paenibacillus*. *Plant Soil* 405(1):125–140. <https://doi.org/10.1007/s11104-015-2526-1>

- Sánchez-López AS, Thijs S, Beckers B, González-Chávez MC, Weyens N, Carrillo-González R, Vangronsveld J (2017) Community structure and diversity of endophytic bacteria in seeds of three consecutive generations of *Crotalaria pumila* growing on metal mine residues. *Plant Soil* 422(1–2):51–66. <https://doi.org/10.1007/s11104-017-3176-2>
- Sarhan MS, Hamza MA, Youssef HH, Patz S, Becker M, El-Sawey H, Nemr R, Daanaa HA, Mourad EF, Morsi AT, Abdelfadeel MR, Abbas MT, Fayed M, Ruppel S, Hegazi NA (2019) Culturomics of the plant prokaryotic microbiome and the dawn of plant-based culture media - A review. *J Adv Res* 19:15–27. <https://doi.org/10.1016/j.jare.2019.04.002>
- Schmidt-Adam G, Gould KS, Murray BG (1999) Floral biology and breeding system of *Opuntia stricta* (Cactaceae). *N Z J Bot* 37(4):687–702. <https://doi.org/10.1080/0028825x.1999.9512663>
- Schmidt-Adam G, Gould KS, Murray BG (2002) Seed biology of *Metrosideros excelsa* (Myrtaceae). *N Z J Bot* 40(3):419–425. <https://doi.org/10.1080/0028825x.2002.9512803>
- Senthilkumar M, Pushpakanth P, Arul Jose P, Krishnamoorthy R, Anandham R (2021) Diversity and functional characterization of endophytic *Methylobacterium* isolated from banana cultivars of South India and its impact on early growth of tissue culture banana plantlets. *J Appl Microbiol* 131(5):2448–2465. <https://doi.org/10.1111/jam.15112>
- Sergeeva V, Alves A, Phillips AJL (2009) *Neofusicoccum luteum* associated with leaf necrosis and fruit rot of olives in New South Wales, Australia. *Phytopathologia Mediterranea* 48(2):294–298. https://doi.org/10.14601/Phytopathol_Mediterr-2790
- Shahid M, Zeyad MT, Syed A, Singh UB, Mohamed A, Bahkali AH, Elgorban AM, Pichtel J (2022) Stress-Tolerant endophytic isolate *Priestia Aryabhatai* BPR-9 modulates Physio-Biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. *Int J Environ Res Public Health* 19(17):10883. <https://doi.org/10.3390/ijerph191710883>
- Sharma A, Song XP, Singh RK, Vaishnav A, Gupta S, Singh P, Guo DJ, Verma KK, Li YR (2022) Impact of carbendazim on cellular growth, defence system and plant growth promoting traits of *Priestia megaterium* ANCB-12 isolated from sugarcane rhizosphere. *Front Microbiol* 13:1005942. <https://doi.org/10.3389/fmicb.2022.1005942>
- Sharma P, Suman A, Aswini K, SaiPrasad J, Gond S (2023) Endophytic bacterial taxonomic and functional diversity in the seeds of wheat genotypes from different agroecologies. *J Plant Interact* 18(1). <https://doi.org/10.1080/17429145.2023.2227652>
- Sheibani-Tezerji R, Naveed M, Jehl MA, Sessitsch A, Rattei T, Mitter B (2015) The genomes of closely related *Pantoea ananatis* maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. *Front Microbiol* 6:440. <https://doi.org/10.3389/fmicb.2015.00440>
- Shetty KG, Minnis AM, Rossman AY, Jayachandran K (2011) The Brazilian peppertree seed-borne pathogen, *Neofusicoccum batangarum*, a potential biocontrol agent. *Biol Control* 56(1):91–97. <https://doi.org/10.1016/j.biocontrol.2010.09.016>
- Simpson P (2005) *Pōhutukawa & rātā*: New Zealand's iron-hearted trees. Te Papa, Wellington
- Singh RR, Wesemael WML (2022) Endophytic *Paenibacillus polymyxa* LMG27872 inhibits *Meloidogyne incognita* parasitism, promoting tomato growth through a dose-dependent effect. *Front Plant Sci* 13:961085. <https://doi.org/10.3389/fpls.2022.961085>
- Singh RK, Singh P, Li H-B, Song Q-Q, Guo D-J, Solanki MK, Verma KK, Malviya MK, Song X-P, Lakshmanan P et al (2020) Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. *BMC Plant Biol* 20(1):220. <https://doi.org/10.1186/s12870-020-02400-9>
- Smith GR, Ganley BJ, Chagne D, Nadarajan J, Pathirana RN, Ryan J, Arnst EA, Sutherland R, Soewarto J, Houlston G et al (2020) Resistance of New Zealand Provenance *Leptospermum scoparium*, *Kunzea robusta*, *Kunzea linearis*, and *Metrosideros excelsa* to *Austropuccinia psidii*. *Plant Dis* 104(6):1771–1780. <https://doi.org/10.1094/PDIS-11-19-2302-RE>
- Sutherland R, Soewarto J, Beresford R, Ganley B (2020) Monitoring *Austropuccinia psidii* (myrtle rust) on New Zealand Myrtaceae in native forest. *New Z J Ecol* 44(2). <https://doi.org/10.20417/nzj ecol.44.23>
- Toome-Heller M, Ho WWH, Ganley RJ, Elliott CEA, Quinn B, Pearson HG, Alexander BJR (2020) Chasing myrtle rust in New Zealand: host range and distribution over the first year after invasion. *Australas Plant Pathol* 49(3):221–230. <https://doi.org/10.1007/s13313-020-00694-9>
- Torres DE, Rojas-Martinez RI, Zavaleta-Mejia E, Guevara-Fefer P, Marquez-Guzman GJ, Perez-Martinez C (2017) *Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* as potential new fungal antagonists of *Puccinia horiana* Henn., the causal agent of chrysanthemum white rust. *PLoS ONE* 12(1):e0170782. <https://doi.org/10.1371/journal.pone.0170782>
- Ulrich K, Stauber T, Ewald D (2008) *Paenibacillus* — a predominant endophytic bacterium colonising tissue cultures of woody plants. *Plant Cell Tissue Organ Cult* 93(3):347–351. <https://doi.org/10.1007/s11240-008-9367-z>
- van der Walt K, Nadarajan J, Mathew L, Bettoni JC, Souza JA (2023) Advances in cryopreservation of *Syzygium maire* (swamp Maire, Maire tawake) zygotic embryos, a critically endangered tree species endemic to New Zealand. *Front Conserv Sci* 4. <https://doi.org/10.3389/fcosc.2023.1269881>
- Vasanthakumari MM, Shridhar J, Madhura RJ, Nandhitha M, Kasthuri C, Janardhana B, Nataraja KN, Ravikanth G, Uma Shaanker R (2018) Role of endophytes in early seedling growth of plants: a test using systemic fungicide seed treatment. *Plant Physiol Rep* 24(1):86–95. <https://doi.org/10.1007/s40502-018-0404-6>
- Verma SK, White JF Jr. (2019) Seed endophytes: biology and biotechnology. Springer Int Publishing AG. 1st edn. <https://doi.org/10.1007/978-3-030-10504-4>
- Wachowska U, Głowacka K, Mikołajczyk W, Kucharska K (2016) Biofilm of *Aureobasidium pullulans* var. *pullulans* on winter wheat kernels and its effect on other microorganisms. *Microbiology* 85(5):523–530. <https://doi.org/10.1134/s0026261716050192>
- Wakelin SA, Warren RA, Harvey PR, Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biol Fertil Soils* 40(1):36–43. <https://doi.org/10.1007/s00374-004-0750-6>
- Wakelin SA, Gupta VV, Harvey PR, Ryder MH (2007) The effect of *Penicillium* fungi on plant growth and phosphorus mobilization in neutral to alkaline soils from Southern Australia. *Can J Microbiol* 53(1):106–115. <https://doi.org/10.1139/w06-109>
- Wang S, Ji B, Su X, Li H, Dong C, Chen S, Zhu Y, Feng W (2020) Isolation of endophytic bacteria from *Rehmannia glutinosa* Libosch and their potential to promote plant growth. *J Gen Appl Microbiol* 66(5):279–288. <https://doi.org/10.2323/jgam.2019.12.001>
- Wang B, Peng H, Wu W, Yang B, Chen Y, Xu F, Peng Y, Qin Y, Fu P, Lu J (2021) Genomic insights into biocontrol potential of *Bacillus stercoris* LJB506. *3 Biotech* 11(11):458. <https://doi.org/10.1007/s13205-021-03000-6>
- Wang J, Shi H, Lu A (2024) Design, synthesis, and antifungal/antioomycete activities of novel 1,2,4-triazole derivatives containing carboxamide fragments. *J Fungi* 10(2). <https://doi.org/10.3390/jof10020160>
- Waqas M, Guarnaccia V, Spadaro D (2022) First report of nut rot caused by *Neofusicoccum parvum* on hazelnut (*Corylus avellana*) in Italy. *Plant Dis* PDIS10212249PDN. <https://doi.org/10.1094/PDIS-10-21-2249-PDN>

- Weller-Stuart T, De Maayer P, Coutinho T (2017) *Pantoea ananatis*: genomic insights into a versatile pathogen. *Mol Plant Pathol* 18(9):1191–1198. <https://doi.org/10.1111/mpp.12517>
- White JF, Kingsley KL, Butterworth S, Brindisi L, Gatei JW, Elmore MT, Verma SK, Yao X, Kowalski KP (2019) Seed-vectored microbes: their roles in improving seedling fitness and competitor plant suppression. Springer International Publishing, pp 3–20. https://doi.org/10.1007/978-3-030-10504-4_1
- Xu M, Sheng J, Chen L, Men Y, Gan L, Guo S, Shen L (2014) Bacterial community compositions of tomato (*Lycopersicon esculentum* Mill.) seeds and plant growth promoting activity of ACC deaminase producing *Bacillus subtilis* (HYT-12-1) on tomato seedlings. *World J Microbiol Biotechnol* 30(3):835–845. <https://doi.org/10.1007/s11274-013-1486-y>
- Yadav U, Bano N, Bag S, Srivastava S, Singh PC (2022) An insight into the endophytic bacterial community of tomato after spray application of propiconazole and *Bacillus subtilis* strain NBRI-W9. *Microbiol Spectr* 10(5):e0118622. <https://doi.org/10.1128/spectrum.01186-22>
- Yamaji K, Fukushi Y, Hashidoko Y, Yoshida T, Tahara S (2001) *Penicillium* fungi from *Picea glehnii* seeds protect the seedlings from damping-off. *New Phytol* 152(3):521–531. <https://doi.org/10.1046/j.0028-646X.2001.00280.x>
- Yong WTL, Ades PK, Tibbits JFG, Bossinger G, Runa FA, Sandhu KS, Taylor PWJ (2018) Disease cycle of *Austropuccinia psidii* on *Eucalyptus globulus* and *Eucalyptus obliqua* leaves of different rust response phenotypes. *Plant Pathol* 68(3):547–556. <https://doi.org/10.1111/ppa.12959>
- Yousuf J, Thajudeen J, Rahiman M, Krishnankutty S, A PA MHAA (2017) Nitrogen fixing potential of various heterotrophic *Bacillus* strains from a tropical estuary and adjacent coastal regions. *J Basic Microbiol* 57(11):922–932. <https://doi.org/10.1002/jobm.201700072>
- Zacaria Vital T, Roman-Ponce B, Rivera Orduna FN, Estrada de Los Santos P, Vasquez-Murrieta MS, Deng Y, Yuan HL, Wang ET (2019) An endophytic *Kocuria palustris* strain harboring multiple arsenate reductase genes. *Arch Microbiol* 201(9):1285–1293. <https://doi.org/10.1007/s00203-019-01692-2>

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