

# Early Growth Stage Root-Associated Endophytes Isolated From *Ulex Europaeus* L. (Fabaceae) Colonizing Rural Areas in South-Central Chile

Hector Herrera University of La Frontera Alejandra Fuentes University of La Frontera Javier Ortiz University of La Frontera Javiera Soto University of La Frontera Rafael Vorges Da-Silva Valadares Instituto Tecnologico Vale, Rua Boaventura da Silva 955 Christian Salas Centro de Modelación y Monitoreo de Ecosistemas, Facultad de Ciencias, Universidad Mayor Cesar Arriagada (Secar.arriagada@ufrontera.cl) University of La Frontera https://orcid.org/0000-0002-8549-6302

#### **Research Article**

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# Abstract

### Background and aims

*Ulex europaeus* L. (Fabaceae), commonly known as *gorse*, is an invasive woody shrub that easily grows in several locations across the world. However, little is known about the interactions of this invasive species with soil microorganisms and how these microbes can promote reaching rapid grow-rates at early stages of development. We aim at characterizing the endophytic fungal and bacterial microbiota associated with roots of early growth stage *U. europaeus* colonizing native ecosystems in south-central Chile.

### Methods

Root-associated microorganisms were isolated and identified using standard molecular techniques. Furthermore, plant growth-promoting traits were studied and biocontrol activity was assessed to characterize the early growth stage root-associated taxa.

#### Results

Four endophytic fungi belonging to Sordariomycetes and twelve bacteria assigned to Proteobacteria and Actinobacteria were identified as the principal early growth stage root-endophytic taxa. Plant growth-promoting traits were detected in several isolates such as *Fusarium acuminatum* and *Rhodococcus* sp. Besides, some of the isolates such as *Rhodococcus* sp. and *Purpureocillium lilacinum* showed biocontrol potential against phytopathogenic fungi.

#### Conclusions

Our results demonstrate that early growth stage root endophytic taxa associated with *U. europaeus* have beneficial plant growth-promoting traits that can contribute with the rapid growth-rates of the shrub. The interaction with a set of beneficial microorganisms is an additional mechanism to explain the ability of *U. europaeus* for colonizing in various ecosystems.

### Introduction

*Ulex europaeus* (Fabaceae) is a shrub species having remarkable ecological features and having several impacts in human-dominated landscapes. The species reaches about 3 meters in height, is spiny, has yellow flowers, and densely cover extended areas (Gränzig et al. 2021; Quiroz et al. 2009). Besides, *U. europaeus* can fix Nitrogen, grow fast, reproduce by seeds that can persist for several years, and live from sea level up to 4000 m in elevation and at several latitudes (Christina et al., 2020). Nonetheless, *U. europaeus* is classified as an invasive species in several regions of the world (Bowman et al., 2008). Once *U. europaeus* can establish in a location, it can alter the native species diversity, causing loss of threatened species and altering economic activities (Christina et al., 2020; Hornoy et al., 2013).

Norambuena et al. (2001) pointed out that *U. europaeus* was introduced in Chile for using it as a hedge plant in agricultural lands to keep out livestock and as a fodder source.

The capacity of *U. europaeus* to handle stressing conditions, successful germination and rapid biomass production is often linked to the ability of the plants from the Fabaceae family to establish symbiotic associations with arbuscular mycorrhizal fungi and symbiosis with nitrogen (N)-fixing bacteria (Rahayu and Pratiwi, 2020; Toma et al., 2017; Wilgan, 2017). These symbiotic interactions have been reported as key to achieving plant establishment in several stressed environments (Hao et al., 2019; Rahayu and Pratiwi, 2020; Sharma et al., 2020). Successful establishment is also linked to the ability of seeds to survive for long periods in the soil seedbank, with an estimated longevity of 20 years and exceeding 10,000 seeds per m<sup>2</sup> (Broadfield and McHenry, 2019). One of the most common management strategies for *U. europaeus* in rural areas of south-central Chile is controlled burn. However, exposure of the soil seedbank to fire can significantly increase seed germination of *U. europaeus*, producing intensive regeneration of new plants from the soil seedbank (Broadfield and McHenry, 2019).

Almost all plant species can interact with endophytic microorganisms as a way to improve fitness to several stressed environments (Fuentes et al., 2020; Lata et al., 2018). Endophytes are a group of bacterial and fungal strains that can live asymptomatically inside plant tissues without causing symptoms of disease (Eid et al., 2019; Herrera et al., 2019a). However, the specific benefits of several endophytic microorganisms for the associated plants are mainly unknown (Bamisile et al., 2018; Ortiz et al., 2019). Recent studies have demonstrated that root endophytes can produce metabolites with antifungal properties to avoid pathogen infection of young seedlings (Terhonen et al., 2016), help wild plants to increase growth (Herrera et al., 2020b), improve tolerance to abiotic stress (Lata et al., 2018), reduce herbivory (Bamisile et al., 2018) or protect against oxidative burst (Khare et al., 2018). Therefore, due to its rigorous invasiveness, it is expected that specific interactions with soil microorganisms at early developmental stages can contribute to the rapid growth and development of this invasive shrub.

The aim of this study was to isolate root-associated microorganisms from *U. europaeus* plantlets colonizing native ecosystems in south-central Chile and to screen for their plant growth promoting capabilities. As far as we know, this is the first study of early growth stage endophytic interactions of the invasive shrub *U. europaeus* colonizing ecosystems in the southern hemisphere.

### **Materials And Methods**

Early growth stage *U. europaeus* plantlets were sampled in agricultural rural areas in a segment of the Nahuelbuta Mountain Range, south-central Chile (38°34'S 72°56'W). The plantlets (n = 20; 3 cm tall) were found in 4 sampling points 2 months after a cleaning and burning treatment of an agricultural soil. The soil samples were collected at a depth of 20 cm for chemical analysis (Fuentes et al., 2020).

The aerial parts of the plantlets were removed and the roots were intensively washed under running tap water to remove the rhizosphere soil. Then, the roots were deposited in 50 ml Falcon tubes and washed

five times with deionized water in a laminar flow cabinet. The cleaned roots were subsequently deposited in sterile 50 ml Falcon tubes and surface disinfected according to Herrera et al. (2019b), where the roots were immersed in 50 ml of a disinfection solution (30 ml of sterile distilled water, 10 ml of sodium hypochlorite and 10 ml of 100 % alcohol) for 5 min, followed by ten washes in sterile deionized water. An aliquot of the last wash was plated in potato dextrose agar (PDA) and Luria Bertani agar (LBA) to rule out the presence of rhizospheric microorganisms in root surface. Six surface-sterilized root segments were placed in Petri dishes containing PDA media supplemented with streptomycin (100 mg l<sup>-1</sup>), Murashige and Skoog basal medium, oatmeal agar (4 g of oats l<sup>-1</sup>, 10 g of agar, pH 5.6) supplemented with benomyl (4 mg l<sup>-1</sup>) to reduce growth of ascomycetes (Bruzone et al., 2015), and LBA supplemented with cycloheximide (100 mg l<sup>-1</sup>). The Petri dishes were incubated in darkness at room temperature until no new fungal and bacterial colonies were detected. Individual bacterial and fungal strains were purified in LBA and PDA, respectively, and classified according their phenotypic characteristics (i.e., growth rate, color, texture, colony border). Purified colonies were stored in individual plates at 4°C and periodically subcultured.

Liquid cultures of the purified fungal and bacterial strains were performed in potato dextrose broth (PDB) and Luria Bertani broth (LBB) respectively to perform DNA extraction. For fungi, six fungal squares (~ 0.5 cm) were inoculated in 100 ml Erlenmeyer flasks containing 40 ml of PDB and incubated for 3 weeks in darkness at room temperature and in an orbital shaker at 150 rpm. An aliquot of 8 ml of medium containing fungal mycelia was taken to store purified strains at -80°C in glycerol. The rest of the media were filtered and the mycelia were used for DNA extraction using the E.Z.N.A.® HP Fungal DNA Kit (Omega Bio-tek, Norcross, GA, USA), according to the manufacturer's recommendations. For bacteria, the purified strains were cultured in 15 ml sterile Falcon tubes containing 5 ml of LBB and cultured in darkness at room temperature and in an orbital shaker at 150 rpm. An aliquot of 800 µl was taken to store the purified strains at -80°C in glycerol. DNA extraction was performed from 1 ml of the liquid culture using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's recommendations. DNA integrity was checked in a 1 % agarose gel, quantified using the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and standardized to 20 ng  $\mu$ l<sup>-1</sup>.

The molecular identification of fungal strains was performed based on the nucleotide sequence of the internal transcribed spacers of the 18S rRNA gene, amplified by PCR using the ITS1 and ITS4 primers (White et al., 1990) following the PCR conditions reported in Herrera et al. (2020b). Similarly, bacterial strains were identified based on the partial 16S rRNA gene sequence, amplified by PCR using the 27F and 1942R primers (Miller et al., 2013) according to the PCR conditions detailed in Herrera et al. (2020a). The PCR amplicons were checked in a 1.5 % agarose gel, quantified in a Qubit fluorometer (Thermo Fisher Scientific) and sequenced at Macrogen (Seoul, South Korea). The nucleotide sequences were compared with those in the GenBank database of the National Center for Biotechnology using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), accepting genus at an identity match greater than 95 % and species at an identity greater than 99 %, as suggested by Chen et al. (2011). The sequences were aligned using the ClustalX software with default conditions for gap opening and gap extension penalty (Larkin et

al., 2007) and non-conserved regions were removed using the BioEdit software v7.2 (Hall, 1999). Operative taxonomic units (OTUs) were assigned at 97 % sequence similarity. The nucleotide sequences obtained were submitted to the GenBank database under the codes MW599973 to MW599982 for bacteria and MW604808 to MW604810 for fungi.

Screening of plant growth-promoting traits of the root-associated microorganisms was performed following standard procedures. The capacity to utilize tricalcium phosphate on agar, indole acetic acid (IAA) production and siderophore production were screened as reported in Soto et al. (2019). Briefly, microorganisms were assayed on Pikovskaya agar plates and incubated in darkness at 26 ± 2°C for 7 days. A clear halo around cultures indicated solubilization of tricalcium phosphate ( $Ca_3(PO_4)_2$ ). For IAA, microorganisms were cultured in LB or potato dextrose broth 1/7 strength, supplemented with 0.5 mg ml  $^{-1}$  of L- tryptophan and then incubated in darkness, under stirring at 150 rpm and 26 ± 2°C for 5 days. After incubation, Salkowski's reagent was added to the cell suspension and measured at 530 nm in a BK-UV1800 spectrophotometer (Biobase, Jinan, China). To determine siderophore production, the isolates were cultured in chrome azurol S (CAS) agar for 5 days, and CAS reaction was determined by color change in the blue CAS agar. Production of exopolysaccharides (EPS) was evaluated following Freeman et al. (1989), where isolates were streaked onto Congo red agar plates and incubated in darkness at 26 ± 2°C for 48 hr. The EPS production was detected by variation in colony color. For ammonia production, microorganisms were grown in 4 % peptone broth and incubated for 7 days in darkness under stirring at 28 ± 2°C. After incubation, Nessler's reagent was added to cell suspension and measured at 450 nm in a BK-UV1800 spectrophotometer (Biobase) (Bhattacharyya et al., 2020). The 1-aminocyclopropane-1carboxylic acid (ACC) deaminase activity was measured according to the free living bacteria method described by Brígido et al. (2015). Briefly, isolates were cultured in tryptic soy broth overnight in darkness, at 26 ± 2°C and 150 rpm, and then collected by centrifugation. The cell pellet was washed twice with Dworkin and Foster (DF) salts minimal medium (without a N source) and re-suspended in DF salt minimal medium with 3 mM ACC for 24 h. Cultures were collected by centrifugation and the cell pellet was used for enzyme activity. The absorbance was measured at 540 nm in a BK-UV1800 spectrophotometer (Biobase). Finally, a biocontrol assay was performed to evaluate the potential of the isolates against phytopathogenic fungal species (Fusarium oxysporum, Botrytis cinerea, Rhizoctonia solani, Phoma herbarum) following Jamali et al. (2020). Briefly, a 5 mm diameter disk of a fresh culture of phytopathogenic fungi was placed in the center of a nutrient agar-potato dextrose agar (1:1) mix plate. Then, bacterial isolates were streaked on both sides of the fungal inoculum at a similar distance of 25 mm and incubated for 7 days at 28 ± 2°C in darkness. For fungi, 5 mm diameter mycelia disks were placed at both sides of the phytopathogenic strains at a similar distance of 25 mm and incubated for 7 days at 28 ± 2°C in darkness. The percentage of inhibition was calculated using the following formula:

% Inhibition = 
$$\left(\frac{C-T}{C}\right) x 100$$

where C is growth in mm in the control and T is growth in mm in the treatment with the isolates.

The quantitative data were analyzed by one-way ANOVA, establishing significant differences at p < 0.05. Post hoc pairwise comparisons were performed using Tukey's multiple range test. All statistical tests were conducted using the R software (R Core Team 2018; https://www.R-project.org).

# Results

The soil chemical analysis showed that the sampling sites had similar content of nitrogen, phosphorous, potassium soil organic matter and pH ranging from 5.3 to 5.8. High extractable aluminum levels were detected in the sampling points, with values ranging from 386 to 526 mg kg<sup>-1</sup> (Table 1).

	Sampling point 1	Sampling point 2	Sampling point 3	Sampling point 4			
N <sup>a</sup>	9	11	12	11			
P <sub>Olsen</sub> <sup>a</sup>	2	2	2	2			
K <sup>a</sup>	356	375	325	442			
Organic matter <sup>b</sup>	5	5	5	4			
Kc	0.91	0.96	0.83	1.13			
Na <sup>c</sup>	0.12	0.10	0.07	0.10			
Ca <sup>c</sup>	8.85	5.44	5.39	5.95			
Mg <sup>c</sup>	7.05	5.70	4.90	5.25			
Alc	0.11	1.58	1.22	0.72			
CEC <sup>c</sup>	17.04	13.78	12.41	13.15			
рН <sup>d</sup>	5.8	5.3	5.4	5.5			
Al <sub>extractable</sub> <sup>a</sup>	386	510	526	455			
<sup>a</sup> mg kg <sup>-1</sup> (total contents)							
b %							
<sup>c</sup> meq/100 g							
<sup>d</sup> In H <sub>2</sub> O							

A total of 79 bacterial colonies were isolated from the analyzed roots, which were separated into 12 different strains based on the morphological characteristic of the colonies and growth rate in culture media. The molecular identification of the isolates revealed 9 OTUs, with a dominance of the phyla Proteobacteria and Actinobacteria (Table 2). Specifically, the isolates UB7 (*Novosphingobium* sp.), UB9 (*Herbaspirillum* sp.), UB2 (*Paraburkholderia strydomiana*), UB4 (*Pseudomonas* sp.) and UB10 (*Herbaspirillum rhizosphaerae*) were assigned to taxa included in the phylum Proteobacteria (Table 2). Similarly, the isolates UB5 (*Rhodococcus* sp.), UB1 (*Terrabacter aerolatus*) and UB11 (*Jatrophihabitans* sp.) were assigned to taxa included in the phylum Actinobacteria (Table 2). The isolates UB3 (*Flavobacterium* sp.) and UB6 (*Paenibacillus odorifer*) were assigned to the phyla Bacteroidetes and Firmicutes, respectively (Table 2). Most of the bacterial sequences were assigned to different OTUs, excluding isolates UB9 and UB10 (*Herbaspirillum* sp.). Isolates UB8 and UB12 were classified as unidentified bacteria (with no significant match in the GenBank database) (Table 2).

#### Table 2

Molecular identification of endophytic microorganisms isolated from young Ulex europaeus plantlets colonizing native ecosystems in south-central Chile

Isolate	Genbank Accesion number	Close relative % identity (Accession number)		Source	Reference
BACTERIA					
UB3	MW599973	<i>Flavobacterium</i> sp. (KF999716)	99	<i>Luffa cylindrica</i> fruit	GenBank
UB6	MW599974	<i>Paenibacillus odorifer</i> (MH157242)	99	Glacier	(Sherpa et al., 2019)
UB5	MW599975	<i>Rhodococcus</i> sp. (AB330417)	100	Soil	(Miwa and Fujiwara, 2009)
UB1	MW599976	<i>Terrabacter aerolatus</i> (KF981511)	98	Contaminated soil	GenBank
UB9	MW599977	<i>Herbaspirillum</i> sp. (LC372610)	97	Ectomycorrhizal root tips	(Obase, 2019)
UB7	MW599978	<i>Novosphingobium</i> sp. (MN989139)	95	<i>Raxinus excelsior</i> leaves	(Ulrich et al., 2020)
UB11	MW599981	<i>Jatrophihabitans</i> sp. (MK875945)	99	Plant	GenBank
UB2	MW599979	<i>Paraburkholderia strydomiana</i> (NR164971)	100	Root nodules	(Beukes et al., 2019)
UB4	MW599980	<i>Pseudomonas</i> sp. (MT012080)	100	Unknown	GenBank
UB10	MW599982	<i>Herbaspirillum rhizosphaerae</i> (MF509845)	99	<i>Polygonatum cyrtonema</i> endosphere	GenBank
UB8	-	Unidentified	-	-	-
UB12	-	Unidentified	-	-	-
FUNGI					

Isolate	Genbank Accesion number	Close relative (Accession number)	% identity	Source	Reference
UF1	MW604808	<i>Fusarium acuminatum</i> (MT514385)	100	Soil	GenBank
UF2	MW604809	<i>Purpureocillium lilacinum</i> (MT530235)	100	Unknown	GenBank
UF6	MW604810	<i>Acremonium alternatum</i> (MG807327)	100	Mediterranean Sea	GenBank
UF5	-	Unidentified	-	-	-

A total of 18 fungal strains were isolated, which were classified into 6 different isolates based on the morphological characteristics of the fungal strains. The molecular identification revealed 3 OTUs and showed Ascomycetes as the principal endophytic phylum associated with *U. europaeus*. Specifically, the isolates UF1 (*Fusarium acuminatum*), UF2 (*Purpureocillium lilacinum*) and UF6 (*Acremonium alternatum*) were related to taxa belonging to the phylum Sordariomycetes (Table 2). The isolate UF5 was classified as unidentified endophytic fungus (without significant match in the GenBank database) (Table 2). Isolates UF3 and UF4 did not grow in synthetic media after initial extraction and purification.

The screening of plant growth-promoting traits showed diverse capabilities associated with the different isolates (Table 3). Solubilization of  $Ca_3(PO_4)_2$  in agar was detected only in *P. strydomiana*, whereas for fungi *P. lilacinum* and unidentified fungi (isolate UF5) showed  $Ca_3(PO_4)_2$  solubilization capability (Table 3). Production of EPS was detected in *P. lilacinum*, *P. odorifer*, *P. strydomiana* and unidentified bacteria isolate UB8, whereas siderophore production was detected in *P. strydomiana* and *P. lilacinum* (Table 3). Almost all tested isolates showed ammonia production capability (14 out 16), being significantly high in the fungal isolate *F. acuminatum* (Fig. 1). The IAA production was similar for all bacterial isolates with higher values in *Pseudomonas* sp., whereas for the fungus *F. acuminatum*, it showed the highest production values detected in the study (Fig. 1). The ACC deaminase activity was significantly higher in the unidentified bacteria isolate UB8 (Fig. 1), whereas no ACC deaminase activity was detected for fungi. Biocontrol potential against potential phytopathogenic fungal strains was detected in 6 out of 16 isolates, with inhibition percentages ranging from 65.8 ± 8.7 (*Rhodococcus* sp.) to 11.1 ± 1.9 (*A. alternatum*) (Table 3). Specifically, the isolates *Rhodococcus* sp., unidentified bacteria (isolate UB8), *F. acuminatum* and *P. lilacinum* showed the highest inhibition percentages against the phytopathogenic fungi (Table 3).

#### Table 3

Plant growth-promoting traits and biocontrol activity of endophytic microorganisms isolated from young Ulex europaeus plantlets. Values are expressed as means ± standard deviation with n = 3. – means no activity, + means positive activity, and + + means high production. Column values with the same letter are not significantly different according to Tukey's multiple range test (p < 0.05).

Isolate	P <sup>a</sup>	EPS <sup>b</sup>	SID <sup>c</sup>	Biocontrol <sup>d</sup>			
				Botrytis cinerea	Rhizoctonia solani	Phoma herbarum	Fusarium oxysporum
Bacteria							
<i>Flavobacterium</i> sp. UB3	-	+	-	-	-	-	-
<i>Paenibacillus odorifer</i> UB6	-	+	-	-	-	-	-
<i>Rhodococcus</i> sp. UB2	-	-	-	65.8 ± 8.7 c	13.9±6.1 a	48.9±1.8 c	60.2 ± 5.8 c
<i>Terrabacter aerolatus</i> UB1	-	-	-	-	-	-	-
<i>Herbaspirillum</i> sp. UB9	-	-	-	-	-	-	-
<i>Novosphingobium</i> sp. UB7	-	-	-	-	-	-	-
<i>Jatrophihabitans</i> sp. UB11	-	-	-	-	-	-	-
Paraburkholderia strydomiana UB2	++	+	+	-	-	-	-
<i>Pseudomonas</i> sp. UB4	-	-	-	-	-	-	-
<i>Herbaspirillum rhizosphaerae</i> UB10	-	-	-	-	-	-	-
Unidentified bacteria isolate UB8	-	+	-	61.4 ± 9.3 bc	24.4±6.1 a	53.7 ± 5.7 c	55.4 ± 3.0 c
Unidentified bacteria isolate UB12	-	-	-	-	-	-	-
Fungi							
<i>Fusarium acuminatum</i> UF1	-	-	-	48.2 ± 1.2 ab	53.6 ± 2.1 c	51.2 ± 2.8 c	58.2 ± 1.5 c
<i>Purpureocillium lilacinum</i> UF2	+	-	+	38.8± 3.1 a	46.8±0.7 bc	25.9 ± 8.5 ab	33.8 ± 0.8 b
<i>Acremonium alternatum</i> UF6	-	-	-	33.3 ± 3.8 a	26.6±1.8 a	11.1 ± 1.9 a	24.0 ± 3.5 a

Isolate	P <sup>a</sup>	EPS <sup>b</sup>	SID <sup>c</sup>	Biocontrol <sup>d</sup>			
				Botrytis cinerea	Rhizoctonia solani	Phoma herbarum	Fusarium oxysporum
Unidentified fungal isolate UF5	+	-	-	45.5± 1.4 ab	41.7 ± 1.2 b	29.0 ± 3.9 b	57.8±0.8 c
<sup>a</sup> Phosphate solubilization							
<sup>b</sup> Exopolysaccharide production							
<sup>c</sup> Siderophore production							
<sup>d</sup> Inhibition percentages							

## Discussion

Our study characterized bacterial and fungal communities associated to the early growth stage of *U. europaeus* growing in rural areas in south-central Chile. Plant growth-promoting traits and biocontrol potential of the isolates were demonstrated, providing novel evidence about beneficial interactions with endophytic microorganisms.

Despite prescribing burning and brush clearing are commonly used for the control of *U. europaeus*, high germination rates from the soil seed bank and rapid plantlet growth after burn have been documented (Broadfield and McHenry, 2019; Madrigal et al., 2012). It is supposed that at this early growth stage endophytic microorganisms can play crucial roles in the establishment of *U. europaeus*, contributing to successful invasiveness in prone areas. Our study provides evidence about several beneficial endophytes living in symbiosis with *U. europaeus* plantlets, some of which may contribute with nutrient solubilization (e.g., *P. strydomiana*, *P. lilacinum*), production of plant growth-regulators (e.g., *Pseudomonas* sp., *T. aerolatus*), and biocontrol potential (e.g., *F. acuminatum*, *Rhodococcus* sp.). These results are in line with recent studies analyzing the beneficial effects of microbial endophytes on growth-promotion and development of native species and those of agricultural interest (Durán et al., 2014; González-Teuber et al., 2017). Therefore, endophytic plant growth-promoting microorganisms can also be essential components at first growth stages of *U. europaeus*.

Early growth stage microbial interactions can play key roles in the establishment of invasive species in the ecosystems, contributing to stress tolerance, plant nutrition and development of the associated plants (Links et al., 2014; Rezki et al., 2018). In our study, we characterized a set of beneficial endophytes associated with initial developmental stages of *U. europaeus* plantlets, but the source of those microbial strains is unclear. Several microbial strains can be stimulated from the soil by root exudates or can be a component of the seed-associated microbiome, both influencing the seedling survival, plant health and productivity (Nelson, 2018). Both, soil-borne and seed-associated microbes can be a potential source of the different taxa identified in our study, but further studies are necessary to define if *U. europaeus* can

associate with soil microorganisms without specificity or if these microbes are mainly seed-associated endophytes. Despite the source of such beneficial microbial taxa, our results suggest that the presence of multiple microbial endophytes with different plant growth-promoting traits can be one of the mechanisms explaining the high growth rates and effective establishment of *U. europaeus*. In fact, Pitzschke (2018) reported that microbial endophytes contribute to a rapid seed germination and plantlet development of *Chenopodium quinoa* even under harsh environmental conditions, which is in line with our results revealing diverse beneficial endophytes colonizing early growth stage *U. europaeus* plantlets. Indeed, microbial endophytes have commonly been detected in association with invasive species, where a key role of such endophytic taxa has been suggested for plant growth promotion, stress tolerance and herbicide resistance of invasive species (Sorty et al., 2016; Suryanarayanan, 2019; Vila-Aiub et al., 2003). Recently, Currie et al. (2020) analyzed and described endophytic fungal interactions of the invasive weed *Impatiens glandulifera*, where the presence of endophytic taxa acts as a barrier that limits the effectivity of biological control strategies. Thus, knowledge about microorganisms inhabiting the endosphere of invasive plants can provide essential information to understand the mechanisms underlining the invasiveness of species such as *U. europaeus*.

Plants from the Fabaceae family usually establish symbiosis with arbuscular mycorrhizal fungi and Nfixing bacteria, which can confer nutritional and physiological benefits on mature plants. As our study was based on culture-dependent methods, we only identified the N-fixing nodulating bacterial genus Paraburkholderia, which has been described as a beneficial taxon associated with Mimosa pudica plants (Paulitsch et al., 2020). Such symbiotic microbial taxa have commonly been described as beneficial microorganisms, but other endophytic strains can also contribute to a successful plant establishment, especially at the beginning of the U. europaeus life cycle. In this sense, we have provided evidence about beneficial attributes of Fusarium endophytic strains associated with U. europaeus in biological control of phytopathogenic fungi and plant-growth promotion. Such fungal genus has commonly been reported as a plant pathogen, but recently the beneficial roles of *Fusarium* spp. in plant growth and development have come to be better understood (Jiang et al., 2019). Similarly, our results showed a strong production of plant-growth regulators by the endophytic strains Herbaspirillum spp. and Pseudomonas sp., which is in line with the studies by Ramos et al. (2020) and Chu et al. (2019) on Oryza sativa and Arabidopsis thaliana, respectively. Additionally, other commonly accepted plant growth-promoter genera were also detected in our analyses, such as Paenibacillus (Bakaeva et al., 2017), Paraburkholderia (Zuñiga et al., 2017), Novosphingobium (Rangjaroen et al., 2017), Flavobacterium (Menon et al., 2020) and *Purpureocillium* (Baron et al., 2020). Therefore, a set of microbial endophytes with multiple plant-growth promoting traits can positively influence the life cycle and physiological responses of young *U. europaeus* plants.

Biocontrol activity is an indirect benefit for plants hosting microbial endophytes. Our results detected *F. acuminatum*, unidentified bacteria (strain UB8) and *Rhodococcus* sp. as the microbial strains with the highest growth inhibition percentages. In fact, Clark et al. (2018) tested the biocontrol potential of a *F. acuminatum* strain isolated from a medicinal plant against *Mycobacterium tuberculosis*, showing that essential metabolites producing by the fungus were directly involved in the antimicrobial activity.

Similarly, our results agree with Hormazabal and Piontelli (2009), who showed that crude extracts from endophytic fungal strains (including the genus *Acremonium*) have biocontrol activity against phytopathogenic fungal strains. Similarly, Vidal et al. (2020) also isolated and characterized endophytic fungi associated with the native plant *Acacia caven* and tested against *B. cinerea*. Regarding bacteria, strong biocontrol potential was detected in the isolate *Rhodococcus* sp., which agrees with Munaganti et al. (2015), who had described the biocontrol activity of a *Rhodococcus* strain against *Pseudogymnoascus destructans*. Similarly, we identified the bacterial genus *Pseudomonas*, which has been described as a bacterium with the ability for biocontrol of fungi (Rojas-Solís et al., 2018). Such increasing evidence about the biocontrol potential of endophytic strains may represent an opportunity to study the mechanisms underlining the high resistance of invasive species to phytopathogens. Therefore, the biocontrol potential can be an indirect mechanism by which endophytic microbial strains contribute to the successful and rapid growth of *U. europaeus* plantlets.

Andisol soils, where *U. europaeus* was sampled, have high levels of available aluminum that interfere with the normal growth and development (Mora et al., 2017). However, such high levels of aluminum seem not to be a problem for *U. europaeus* plantlets. It is expected that effective tolerance mechanisms can support colonization of acidic Andisols. One of the tolerance mechanisms can be related with specific interactions with microbial strains which can confer metal tolerance to their associated plants (Ortiz et al., 2019). This is the case of *Rhodococcus* sp., which has been described as a microbial strain with high tolerance to metal(loid)s (Kumari et al., 2019; Vergani et al., 2019). Such metal tolerance conferred by endophytic strains can have a positive role in the tolerance of phytotoxic aluminum levels for *U. europaeus* plants inhabiting acidic Andisols.

Our study results provide evidence of novel endophytic interactions of *U. europaeus* with soil-borne microorganisms, from which their plant growth-promoting traits as well as their biocontrol potential can be an additional mechanism to explain the high growth rates and establishment of *U. europaeus*. However, it is unclear if the high diversity of endophytic interactions is induced by the ability of *U. europaeus* to select beneficial soil microorganisms from the bulk soil, or if the microbial strains are part of the seed-associated microbiome. Additionally, microbial strains with multiple beneficial traits can be considered potential bioinoculants for improving the plant growth or screening for novel biocontrol strategies of phytopathogenic fungi.

# Declarations

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### **Declaration of Competing Interest**

The authors declare no conflict of interest.

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# Figures



### Figure 1

Ammonia production (a), indoleacetic acid (IAA) production (b) and 1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity (c) of endophytic microorganisms isolated from young Ulex europaeus

plantlets growing in south-central Chile. Values are expressed as means  $\pm$  standard deviation with n= 3 and bars with the same letter are not significantly different according to Tukey's multiple range test (p <0.05).