Plant Soil
https://doi.org/10.1007/s11104-022-05324-5

REGULAR ARTICLE

Root-associated endophytes isolated from juvenile *Ulex europaeus* L. (Fabaceae) plants colonizing rural areas in South-Central Chile

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Received: 11 August 2021 / Accepted: 30 January 2022

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 rapid grow-rates at early stages of development. We aim to explore this by characterizing the endophytic fungal and bacterial microbiota associated with roots of juvenile *U. europaeus* plants colonizing native ecosystems in south-central Chile.

Methods Root-associated microorganisms were isolated and identified using standard molecular techniques. Furthermore, plant growth-promoting traits and biocontrol activity of the isolates against phytopathogenic fungi were 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 associated with the early stage of *U. europaeus*. Plant growth-promoting traits were detected in some isolates such as *Fusarium acuminatum* and *Rhodococcus* sp. Additionally, two endophyte isolates *Rhodococcus* sp. and *Purpureocillium lilacinum* showed biocontrol potential against phytopathogenic fungi tested in this study.

Conclusions Our results demonstrate that *U. europaeus* plantlets host several endophytes in the roots, some of which showed biocontrol capabilities and plant growth-promoting traits that can contribute with the rapid growth-rates at juvenile stages of the shrub. The interaction with a set of endophytes demonstrating these beneficial traits is an additional mechanism explaining the ability of *U. europaeus* to colonize various ecosystems.

Keywords Bacteria · Endophytes · Fungi · Invasive species · Plant growth-promotion · Symbiosis · Biocontrol

Responsible Editor: Jeff R. Powell.

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Published online: 17 February 2022

Introduction

Ulex europaeus L. (Fabaceae), commonly known as *gorse*, is a common invasive woody weed that colonizes a wide range of geographical habitats, including the ecosystems of the southern Andes (Gränzig et al. 2021). This species has been introduced in agricultural landscapes to keep livestock and as a fodder source (Norambuena et al. 2001). However, its widespread occurrence has been recognized as a serious environmental issue (Hornoy et al. 2013). Once *U. europaeus* can effectively establish in a microhabitat, it can significantly alter the native species diversity and economic activities (Christina et al. 2020; Hornoy et al. 2013). *U. europaeus* has many traits which make it an effective colonizer in many ecosystems; it is a species that advances over the native plants spreading its seeds and branches and only in a few years it manages to invade large areas, moving away not only plant species, but also the associated fauna that lives in the native forest (Hill et al. 2008). Additionally, the species has an extended flowering period, can establish symbiosis with beneficial soil microorganisms and produce seeds even under severe environmental conditions (Bowman et al. 2008; Christina et al. 2020) and the seeds can survive for long periods in the soil seed bank, with an estimated longevity of 20 years and exceeding 10,000 seeds per m^2 (Broadfield and McHenry 2019). Controlled burning is one of the most common management strategies for *U. europaeus* in rural areas of south-central Chile. However, exposure of the soil seed bank to fire can significantly increase seed germination, producing intensive regeneration of new plantlets from the soil (Broadfield and McHenry 2019). Moreover, this strategy can alter soil chemistry, microbial communities and nutrient cycling and can induce severe changes in microbial diversity (Lucas-Borja et al., 2019). After germination, the interaction of juvenile plants with beneficial fungi and bacteria can be key to improve the growth rates and avoid the infection by pathogenic fungi.

Endophytes are a group of microorganisms (bacteria, fungi, archaea and virus) that reside inside the plant tissues (Eid et al. 2019; Fadiji et al. 2020; Nerva et al. 2017) some of which can improve plant fitness in harmful environments (Lata et al. 2018). However, the specific benefits of several plant endophytes 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 by competing with phytopathogens or by directly antagonism through the production of antimicrobial compounds (Bolívar-Anillo et al. 2020; Terhonen et al. 2016). Additionally, endophytes can help improve plant tolerance to abiotic stress (Lata et al. 2018), increase growth through the production of metabolites such as indoleacetic acid (IAA), siderophores, ACC deaminase, phosphate solubilization and exopolysaccharides production (Soto et al. 2019), reduce herbivory by the production of toxic compounds that make plants less desirable (Bamisile et al. 2018; Hartley and Gange 2009), and protect against oxidative stress (Khare et al. 2018). Therefore, due to the rigorous invasiveness of *U. europaeus*, it is expected that specific interactions with microbial endophytes at early developmental stages can contribute to the rapid growth and development of this shrub.

Research suggests that interactions with beneficial microorganisms (i.e. arbuscular mycorrhizal fungi and nitrogen (N)-fixing bacteria) is crucial to help plants to successfully germinate, grow, and handle stressing environmental conditions (Hao et al. 2019; Rahayu and Pratiwi 2020; Wilgan 2021). However, the symbiosis with other microbial taxa can also have positive effects on plant nutrition, protection against phytopathogens and invasiveness (Jeong et al. 2021; Soares et al. 2016), an issue that has been scarcely explored in invasive shrubs. These strains with beneficial effects on growth of invasive species can also be explored to develop bioinoculants to improve growth of agricultural or forestry plants. Therefore, understanding why this species is a particularly good invader of many habitats and how it interacts with microorganisms is foundational to know the mechanisms that promote the invasion in native landscapes.

The aim of this study was to isolate root-associated endophytes from *U. europaeus* plantlets colonizing rural areas in south-central Chile and to screen for their plant growth promoting traits and biocontrol potential against phytopathogenic fungi. As far as we know, this is the first study of endophytic interactions of the invasive shrub *U. europaeus* colonizing ecosystems in the southern hemisphere. Knowledge about endophytes associated with invasive species is crucial to understand the life cycle of these species and

explore the mechanisms that contribute with the rapid growth and high establishment rates.

Materials and methods

Juvenile *U. europaeus* plants were sampled in an agricultural rural area in the Nahuelbuta Mountain Range, south-central Chile (38°33′59″S: 72°56′37″W). The plantlets (n = 20; 3 cm tall) were collected at each of the four sampling sites, 2 months after a cleaning and burning treatment. Additionally, 500 g of bulk soil samples were collected at a depth of 0-20 cm for chemical analysis following Fuentes et al. (2020). Briefly, the available P content was determined by extraction with $NaHCO_3$ at pH 8.5 and the available K was determined according to Mingorance (2002). The organic matter content was determined by the method described by Walkley and Black (1934). The pH was measured using 1:2.5 ratio of soil:deionized water. Cation exchange capacity (CEC) was calculated from the total exchangeable bases (Mg, Ca, K, and Na extracted by 1 M ammonium acetate at pH 7.0) analyzed by flame atomic absorption spectrophotometry, according to Sadzawka et al. (2004).

The above-ground 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. (2019), where the roots were immersed in 50 ml of a disinfection solution (30 ml of sterile distilled water, 10 ml of sodium hypochlorite (0.5% active chlorine) and 10 ml of 100% alcohol) for 2 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 the root surface. Six 0.5 cm surface-sterilized root segments were placed in each Petri dish containing PDA media supplemented with streptomycin (100 mg l^{-1}), Murashige and Skoog basal medium, oatmeal agar (4 g of oats l^{-1}, 10 g of agar, pH 5.6) supplemented with benomyl (4 mg l^{-1}) to reduce growth of ascomycetes (Bruzone et al. 2015), and LBA supplemented with cycloheximide (100 mg l^{-1}). The Petri dishes were incubated in darkness at room temperature until no new fungal or 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.

DNA was extracted from pure liquid cultures of fungal and bacterial isolates which had been grown on potato dextrose broth (PDB) and Luria Bertani broth (LBB) media respectively**.** For fungi, six mycelial plugs (~0.5 cm) were inoculated into 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. Fungal and bacterial 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 μl^{-1}.

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% (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 for both, fungi and bacteria. 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 ($Ca_3(PO_4)_2$) 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 $Ca_3(PO_4)_2$. 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 the isolates were streaked onto Congo red agar plates and incubated in darkness at 26 ± 2 °C for 48 h. 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 the cell suspension and measured at 450 nm in a BK-UV1800 spectrophotometer (Biobase) (Bhattacharyya et al. 2020). The 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity for fungal and bacterial isolates was measured according to Brígido et al. (2015), with minor modifications. Briefly, the isolates were cultured in tryptic soy broth overnight in darkness, at 26 ± 2 °C and 150 rpm, and then collected by centrifugation at 5000 rpm. 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 root endophytic fungal and bacterial isolates against phytopathogenic fungi (obtained from the Bioremediation Laboratory of Universidad de La Frontera collection; *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:

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

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^{-1} (Table 1).

Table 1 Chemical characterization of soil colonized by *Ulex europaeus* in south-central Chile

	Sampling point 1	Sampling point 2	Sampling point 3	Sampling point 4
Nitrogen[a]	9	11	12	11
Phosphorus $_{Olsen}$[a]	2	2	2	2
Potassium[a]	356	375	325	442
Organic matter[b]	5	5	5	4
Potassium[c]	0.91	0.96	0.83	1.13
Sodium[c]	0.12	0.10	0.07	0.10
Calcium[c]	8.85	5.44	5.39	5.95
Magnesium[c]	7.05	5.70	4.90	5.25
Aluminum[c]	0.11	1.58	1.22	0.72
Cation Exchange Capacity[c]	17.04	13.78	12.41	13.15
pH[d]	5.8	5.3	5.4	5.5
Aluminum $_{extractable}$[a]	386	510	526	455

[a]mg kg^{-1} (total contents)

[b]%

[c]meq/100 g

[d]In H_2O

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* spp.). Isolates UB8 and UB12 were classified as unidentified bacteria (with no significant match in the GenBank database) (Table 2).

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 main phylum associated with *U. europaeus* plantlets. 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 an 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 isolates (Table 3). Solubilization of $Ca_3(PO_4)_2$ in agar was detected only in the bacterial strain *P. strydomiana*. Similarly, the fungal strains *P. lilacinum* and unidentified isolate UF5 showed $Ca_3(PO_4)_2$ solubilization capability (Table 3). Production of EPS was detected in the fungal strains *P. lilacinum*, *P. odorifer*, and in the bacterial strain *P. strydomiana* and unidentified bacteria isolate UB8. Siderophore production was detected in the bacteria *P. strydomiana* and in the fungus *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

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 (Accession number)	% identity	Source	Reference
Bacteria					
UB1	MW599976	*Terrabacter aerolatus* (KF981511)	98	Contaminated soil	GenBank
UB2	MW599979	*Paraburkholderia strydomiana* (NR164971)	100	Root nodules	(Beukes et al., 2019)
UB3	MW599973	*Flavobacterium* sp. (KF999716)	99	*Luffa cylindrica* fruit	GenBank
UB4	MW599980	*Pseudomonas* sp. (MT012080)	100	Unknown	GenBank
UB5	MW599975	*Rhodococcus* sp. (AB330417)	100	Soil	(Miwa and Fujiwara, 2009)
UB6	MW599974	*Paenibacillus odorifer* (MH157242)	99	Glacier	(Sherpa et al., 2019)
UB7	MW599978	*Novosphingobium* sp. (MN989139)	95	*Raxinus excelsior* leaves	(Ulrich et al., 2020)
UB8	–	Unidentified	–	–	–
UB9	MW599977	*Herbaspirillum* sp. (LC372610)	97	Ectomycorrhizal root tips	(Obase, 2019)
UB10	MW599982	*Herbaspirillum rhizosphaerae* (MF509845)	99	*Polygonatum cyrtonema* endosphere	GenBank
UB11	MW599981	*Jatrophihabitans* sp. (MK875945)	99	Plant	GenBank
UB12	–	Unidentified	–	–	–
Fungi					
UF1	MW604808	*Fusarium acuminatum* (MT514385)	100	Soil	GenBank
UF2	MW604809	*Purpureocillium lilacinum* (MT530235)	100	Unknown	GenBank
UF5	–	Unidentified	–	–	–
UF6	MW604810	*Acremonium alternatum* (MG807327)	100	Mediterranean Sea	GenBank

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 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).

Discussion

In this study we have characterized bacterial and fungal endophytes isolated from *U. europaeus* plantlets growing in rural areas in south-central Chile. Plant growth-promoting traits and biocontrol potential of the isolates were demonstrated in vitro, providing evidence of how endophytic microorganisms contribute to establishment success at early developmental stages.

This study detected several beneficial endophytes living in symbiosis with *U. europaeus* plantlets, some of which contribute to nutrient solubilization (e.g.,

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. – 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[a*]	EPS[b*]	SID[c*]	Biocontrol[d**]			
				Botrytis cinerea	*Rhizoctonia solani*	*Phoma herbarum*	*Fusarium oxysporum*
Bacteria							
Terrabacter aerolatus UB1	–	–	–	–	–	–	–
Paraburkholderia strydomiana UB2	++	+	+	–	–	–	–
Flavobacterium sp. UB3	–	+	–	–	–	–	–
Pseudomonas sp. UB4	–	–	–	–	–	–	–
Rhodococcus sp. UB5	–	–	–	65.8 ± 8.7 c	13.9 ± 6.1 a	48.9 ± 1.8 c	60.2 ± 5.8 c
Paenibacillus odorifer UB6	–	+	–	–	–	–	–
Novosphingobium sp. UB7	–	–	–	–	–	–	–
Unidentified bacteria isolate UB8	–	+	–	61.4 ± 9.3 bc	24.4 ± 6.1 a	53.7 ± 5.7 c	55.4 ± 3.0 c
Herbaspirillum sp. UB9	–	–	–	–	–	–	–
Herbaspirillum rhizosphaerae UB10	–	–	–	–	–	–	–
Jatrophihabitans sp. UB11	–	–	–	–	–	–	–
Unidentified bacteria isolate UB12	–	–	–	–	–	–	–
Fungi							
Fusarium acuminatum UF1	–	–	–	48.2 ± 1.2 ab	53.6 ± 2.1 c	51.2 ± 2.8 c	58.2 ± 1.5 c
Purpureocillium lilacinum UF2	+	–	+	38.8 ± 3.1 a	46.8 ± 0.7 bc	25.9 ± 8.5 ab	33.8 ± 0.8 b
Unidentified fungal isolate UF5	+	–	–	45.5 ± 1.4 ab	41.7 ± 1.2 b	29.0 ± 3.9 b	57.8 ± 0.8 c
Acremonium alternatum UF6	–	–	–	33.3 ± 3.8 a	26.6 ± 1.8 a	11.1 ± 1.9 a	24.0 ± 3.5 a

[a]Phosphate solubilization

[b]Exopolysaccharide production

[c]Siderophore production

[d]Inhibition percentages

*n = 9

**n = 5

P. strydomiana, *P. lilacinum*), produce plant growth-regulators (e.g., *Pseudomonas* sp., *T. aerolatus)*, and have biocontrol potential (e.g., *F. acuminatum, Rhodococcus* sp.). Interactions with fungal endophytes have been previously explored in adult plants of *U. europaeus*, where several taxa were isolated from spines and stems, including endophytic *Fusarium* spp. strains (Fisher et al. 1986; Fisher and Petrini 1988). After a burning and brush clearing treatment, the endophytic microorganisms associated with juvenile plants play crucial roles in the establishment of *U. europaeus,* contributing to its successful invasiveness in prone areas. These results are in line with recent studies analyzing the beneficial effects of microbial endophytes on growth-promotion and development of invasive species (Aschehoug et al. 2012; Graff et al. 2020; Molina-Montenegro et al. 2015). Therefore, endophytic plant growth-promoting microorganisms can also be essential components at first growth stages of *U. europaeus.*

In our study, we characterized a set of endophytes associated with *U. europaeus* plantlets. Microbial

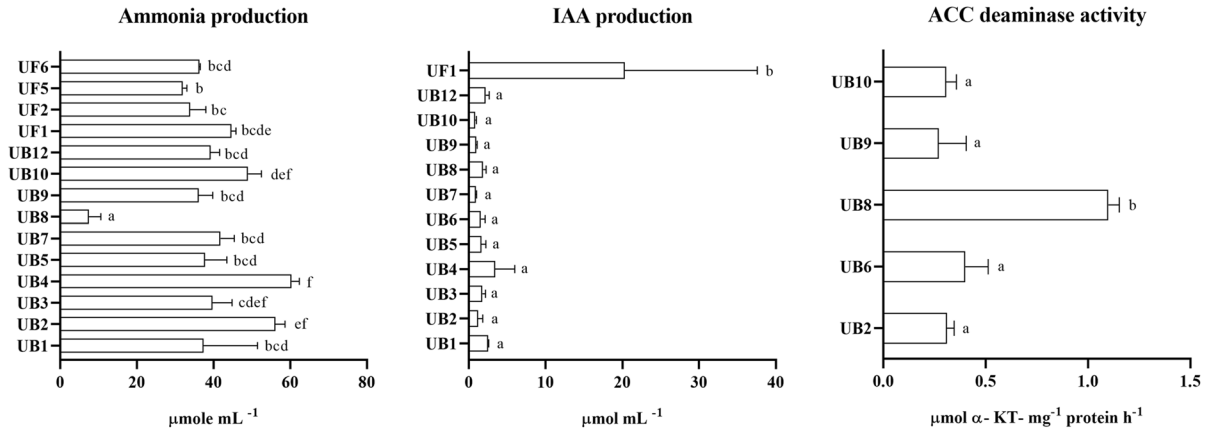

Fig. 1 Ammonia production (**a**), indoleacetic acid (IAA) production (**b**) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (**c**) of endophytic microorganisms isolated from young *Ulex europaeus* plantlets growing in south-central Chile. Values are expressed as means ± standard deviation with n = 5. Bars with the same letter are not significantly different according to Tukey's multiple range test ($p < 0.05$)

interactions play key roles in the establishment of invasive species, contributing to stress tolerance, plant nutrition and development of the associated plants (Links et al. 2014; Rezki et al. 2018). These microbial taxa 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 can be a potential source of the microbial 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.

Our results reveal the presence of multiple endophytes in the roots of juvenile *U. europaeus* plants which exhibit chemical/metabolic properties similar to plant growth-promoting microorganisms. Recent studies report that beneficial microbial endophytes can contribute to a rapid growth and development of invasive species growing even under severe environmental conditions (Jeong et al. 2021; Newcombe et al. 2009). 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 (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 efficacy of biological control strategies. Thus, knowledge about endophytes of invasive plants can provide essential information to understand the mechanisms underlining the invasiveness. However, further greenhouse studies are necessary to define the functional importance of these endophytes in the early growth stages of *U. europaeus*.

Plants from the Fabaceae family establish symbiosis with arbuscular mycorrhizal fungi and N-fixing 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 bacteria associated with *Mimosa pudica* (Paulitsch et al. 2020). Such symbiotic microbial taxa have commonly been described as beneficial symbionts, but other endophytic strains can also contribute to a successful plant establishment, especially at the beginning of the *U. europaeus* life cycle. Additionally, we have provided evidence about beneficial activities of *Fusarium* endophytic strains isolated from *U. europaeus*. This fungal genus has commonly been reported as a plant pathogen, but recently the beneficial roles of *Fusarium* spp. endophytic strains in plant growth and development have come to be better understood (Jiang et al. 2019; Kuldau and Yates 2000). In this sense, recent studies have provided evidence about the beneficial effects of endophytic

Fusarium spp. strains in secondary metabolites with critical roles in the plant response to environmental stresses, which can indirectly influence the naturalization of invasive species (Nieva et al. 2021; Ola et al. 2013). 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, commonly accepted plant growth-promoting genera were also detected in our analyses, including *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 endophytes with biochemical capabilities that benefit the plant can positively influence the growth of *U. europaeus* plantlets. However, further studies must be performed in order to explore each of these mechanisms and understand the efficacy of the isolated endophytes in growth promotion and biocontrol under in-vivo conditions.

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 fungi. Similarly, Vidal et al. (2020) also isolated and characterized endophytic fungi associated with *Acacia caven* and probed their biocontrol capability 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 genera *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 associated with invasive species represent an opportunity to understand the mechanisms underlining the biocontrol of phytopathogens (Mdee et al. 2009; Shuping and Eloff 2017). Therefore, biocontrol 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 of plants (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). Additionally, endophytic strains can influence the metabolic activity of the plant roots enhancing the production of secondary metabolites involved in metal tolerance such as low molecular weight organic acids and phenolic compounds (Chen et al. 2014; Das et al. 2012). Such metal tolerance conferred by endophytic strains have a positive role in the tolerance of phytotoxic aluminum levels for *U. europaeus* inhabiting acidic Andisols.

Our results provide evidence of novel interactions of *U. europaeus* with endophytic microorganisms, from which their plant growth-promoting traits as well as their biocontrol potential are additional mechanisms that can explain the rapid growth rates at early developmental stages of this shrub. Additionally, further work is required to explore the utility of these endophytic strains as potential bioinoculants for improving the growth of native and agricultural plants or controlling against phytopathogenic fungi.

Funding This study was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico of Chile [grant numbers 3200134 and 1211857].

Declarations

Competing interests The authors declare no conflict of interest.

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