



# Endophytic and rhizospheric microbial communities associated with native and introduced cultivated plant species in Uruguay as sources for plant growth promotion bioinoculant development

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## Abstract

The plant holobiont is the sum of the plant and the biological communities that live in and on it. In particular, the plant microbiota is the set of fungal and bacterial communities associated with plants. Different microbiotas are defined by the plant compartments, including the rhizospheric, endophytic, and epiphytic. These plant-microbiota interactions are strong determinants of plant holobiont growth, health, and productivity, and those beneficial microorganisms involved in these processes are known as plant growth-promoting microorganisms (PGPM). The characteristics of the PGPM highlight their potential for biotechnological use in agriculture as bioinputs (biofertilizers, biopesticides, and biostimulants) as an alternative to the indiscriminate use of chemical inputs in agricultural technology packages. The Uruguayan economy is based mainly on livestock and agricultural production. In agricultural systems, the technological packages used imply in most cases the administration of large amounts of chemical inputs. These activities cause serious environmental and economic problems in the country, highlighting the need to develop new and more sustainable agricultural practices. It is in this context where PGPM play a fundamental role. The aim of this review is to summarize the current knowledge regarding the microbiota associated with cultivated and non-cultivated (including native and introduced) plant species grown in Uruguay. In particular, it will focus on the description of the research related to the endophytic and rhizospheric bacterial as well as mycorrhizal interactions, as potential sources for bioinputs development.

**Keywords** Plant holobiont · Plant microbiota · Plant growth promoting microorganisms · Agronomic bioinputs · Uruguay

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## Introduction

The plant holobiont (holo: all, biont: organisms) is the sum of the plant and the biological communities that live in and on it (Rosenberg and Zilber-Rosenberg 2016; Lyu et al. 2021). One of the components of these communities is the microbiota, the set of fungal and bacterial communities (Vandenkoornhuysen et al. 2015; Berg et al. 2020). Plant compartments define different microbiota, among which are the rhizospheric microbiota (associated with the rhizosphere of the roots), the endophytic microbiota (associated with the internal tissues), and the epiphytic microbiota (associated with the surfaces of roots, stems, and leaves) (Gopal and Gupta 2016). Therefore, the plant holobiont phenotype results from the interaction between the plant and all its associated microbiota, expanding the genetic potential of

plants (Compant et al. 2019). In turn, this resulting phenotype is influenced by environmental conditions, including the effect of biotic and abiotic factors, as well as the agricultural practices applied in the case of crops (Cordovez et al. 2019). Particularly in the microbiotas mentioned, there are microorganisms that directly or indirectly have a beneficial effect on the health and development of plants, which are called plant growth-promoting microorganisms (PGPM) (Turner et al. 2013; Etalo et al. 2018). Several direct or indirect mechanisms have been reported in microorganisms as involved in plant growth promotion (PGP) (Compant et al. 2010; Naik et al. 2019; Smith and Read 2008). The direct PGP mechanisms described include the absorption and solubilization of poorly bioavailable minerals for the plants (e.g. Fe, P, K), by bacteria and mycorrhizal fungi (Marschner et al. 2011; Plassard and Bell 2010; Schreider et al. 2022). Likewise, diazotrophic microorganisms are capable of incorporating atmospheric N<sub>2</sub> into the plant, through the biological nitrogen fixation (BNF) process by establishing symbiotic relationships with the hosts. This has been extensively studied in bacteria of the genus *Rhizobium* in symbiosis with legumes, or in bacteria of the genera *Herbaspirillum* and *Azospirillum*, by establishing endophytic interactions with non-legume plants (Wagner et al. 2011). Another direct PGP mechanism described in microorganisms is the production or modulation of phytohormones (auxins, gibberellins, cytokinin) by bacteria associated with plants of the genera *Azospirillum* and *Bacillus*, among others (Zaheer et al. 2022; Etalo et al. 2018). On the other hand, the most studied indirect PGP mechanisms by microorganisms are the biological control of phytopathogens and the induction of systemic resistance in plants by bacteria (Van der Wolf and De Boer 2015). Likewise, microorganisms associated with plants have also been described as involved in the defense against abiotic stress situations such as drought or salinity. This type of mechanism has been widely reported for bacteria and mycorrhizae (Kumar et al. 2020; Smith and Read 2008). The expression of these traits is a multifactorial effect that depends on the specificity of the plant-microorganism interaction, plant genotype, soil type, climate conditions, and agricultural practices (Guttman et al. 2014; Scheres and Van Der Putten 2017; Compant et al. 2019). These characteristics of PGPM highlight their great potential for biotechnological use in agriculture, particularly in the development of bioinputs (biofertilizers, biopesticides, biostimulants). These bioinputs are a viable alternative to the indiscriminate use of chemical inputs in agricultural technology packages, providing economic and environmental sustainability. Current agricultural production systems are mainly based on the use of chemical inputs (e.g., fertilizers, pesticides) to promote the growth and health status of crops. This agricultural practice leads to serious environmental and economic

problems, especially in countries where chemical inputs are imported. From an environmental point of view, it is well known that plants are capable of incorporating only 50% of the supplied chemical fertilizers. The remaining 50% runs off into above and belowground water bodies, causing contamination (Vitousek et al. 1997; Tilman 1998; Castelli et al. 2021; Alcántara et al. 2022; Kruk et al. 2023). In addition, the production of these chemical inputs depends on the consumption of fuel of fossil origin, increasing the carbon footprint. It is in this context that bioinputs based on PGPM become very relevant. The development process of a bioinput takes time and several stages, including the isolation of microorganisms, their identification and characterization in vitro (assessing the presence of PGP characteristics in them) and in vivo (assessing the effectiveness of their inoculation on the plant under different conditions: in vitro, greenhouse and field), as well as its scaling, formulation, and registration. Likewise, studies focused on the microbial ecology of the plant-microbe interaction should be carried out to understand the impact of putative inoculants and agricultural practices on the diversity, composition, and activity of the microbiota associated with the soil and the roots of the plants under study. Uruguay is a pioneer in the formulation and use of bioinputs based on PGPM for different crops. In this sense, studies and developments have been focused mainly on bioinoculants based on bacteria of the genus *Rhizobium*, which establish symbiotic associations with legume plants, to which they supply the necessary N for their growth through the BNF process (Fabiano et al. 2023). Moreover, Uruguay has robust bioinput legislation for its registration and commercialization (Mayans, personal communication, <https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/dgsa>). However, the research and development of bioinputs based on rhizospheric and endophytic PGPM are still incipient. Nonetheless, different efforts have been made in recent decades to study the microbiota associated with native and introduced cultivated plant species grown in Uruguay as a starting point for bioinput development. The aim of this review is to summarize the current knowledge regarding the microbiota associated with cultivated and non-cultivated (including native and introduced) plant species grown in Uruguay. In particular, it will focus on the description of the studies related to the endophytic and rhizospheric bacteria as well as mycorrhizal microbiota, with potential as sources of bioinputs development. The work will be described according to the progress of the research carried out in each of the crops studied.

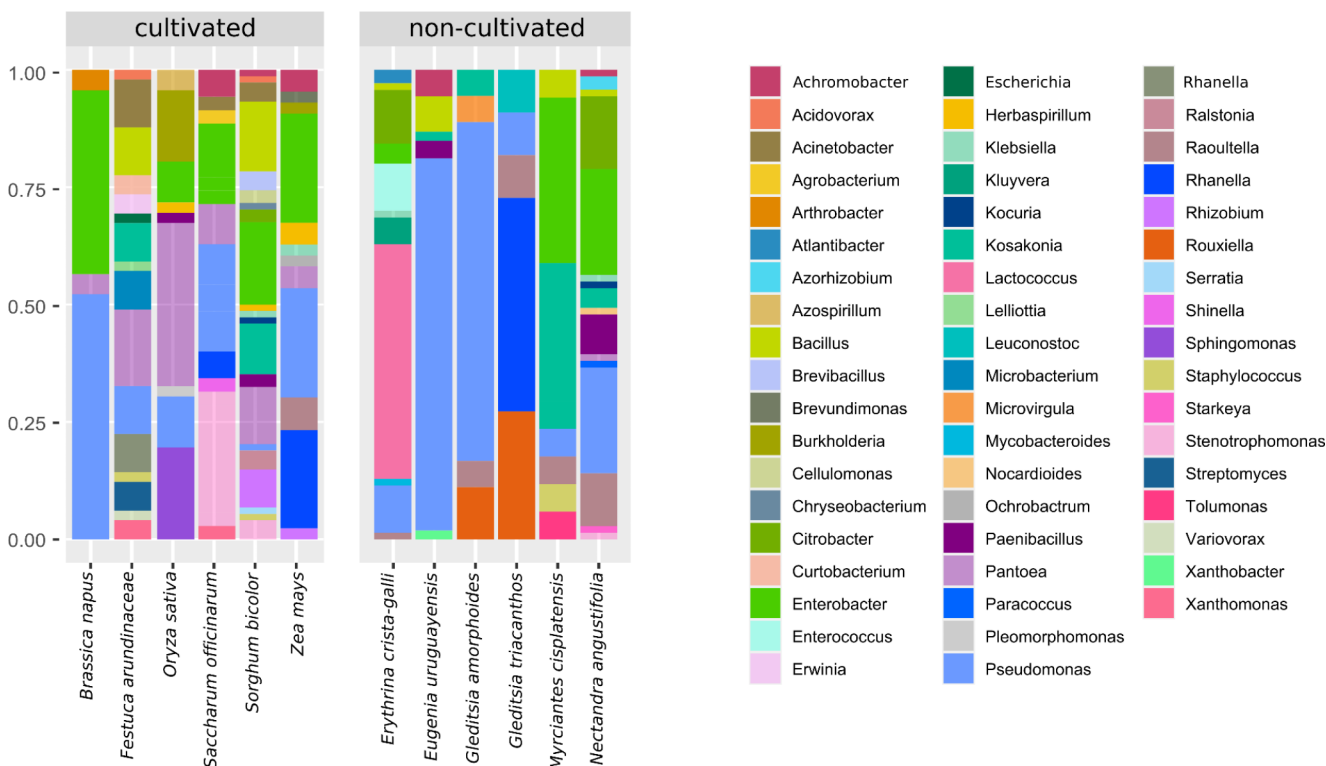
## Cultivated plant species

### Canola (*Brassica napus*)

Canola occupies the second place in global oilseed production and third place in global vegetable oil consumption. This crop belongs to the Brassicaceae family and is used in several regions worldwide for the production of edible fodder, edible oil, and biodiesel. In Uruguay canola occupied 7.4% of the cultivated area in 2021 (MGAP/DIEA 2022); this crop is cultivated for the production of oil and biodiesel, but its optimal growth depends on high amounts of chemical fertilizer inputs. With the aim of developing a future bioinoculant, a collection of 333 putative bacterial endophytes associated with the internal tissues of roots and stems of cultivars Trapper and Igranola103 grown in Uruguay was constructed and characterized in vitro and in vivo (Fig. 1; Table 1). Isolates of the collection with different PGP traits (Fe, P, K solubilization, auxin production, and the potential ability to fix N), were identified and assigned to *Enterobacter*, *Pseudomonas*, *Arthrobacter*, and *Pantoea* genera. Moreover, isolates related to the *Pseudomonas* and *Arthrobacter* genera were described as PGP of the cultivar Igranola103 (Ferrari 2017).

### Tall fescue (*Festuca arundinacea*)

Tall fescue is a non-native grass species (Poaceae family) used for cultivated prairies for cattle feeding (Milne 2010). In particular, the cultivar SFRO Don Tomás (DT) appeared naturally in the Uruguayan fields and is highly tolerant to cold and drought stress. Based on the now widely accepted concept that the microbiome is associated with plant health, development, and stress resistance (Berg et al. 2020), a collection of 342 endophytic bacteria was created from seeds, aerial parts, and roots of the field-grown cultivar SFRO Don Tomás (De los Santos et al. 2015). The functional ability of the isolates to produce IAA, solubilize minerals (P, Fe, K), and perform BNF was determined. In addition, several infection traits, such as the ability to produce proteases, peroxidases, cellulases, and hemicellulases, were identified in the isolates. Furthermore, 33 isolates were taxonomically identified by sequencing of their 16S rRNA genes and shown to belong to a broad phylogenetic spectrum, including the genera *Bacillus*, *Microbacterium*, *Curtobacterium*, *Streptomyces*, *Acidovorax*, *Variovorax*, *Acinetobacter*, *Pseudomonas*, *Pantoea*, *Rhanella* and *Xanthomonas* (Fig. 1). In addition, a group of isolates was tested as an inoculant in PGP assays on two tall fescue cultivars, DT and Tacuabé (Ta). From that test, several isolates were identified as PGP in vitro and in greenhouse conditions (Table 1). Moreover,



**Fig. 1** Relative abundance of bacterial genera of Uruguayan endophytes isolates obtained in different collections. On the left panel, relative abundances in plants of agronomic use (crop), and on the right in

wild plant species. Color codes used for the different genera are the same for both panels

**Table 1** Endophytic and plant associated bacteria with PGP properties studied in Uruguay

Strain	Plant source	Plant growth promotion mechanism (in vitro) <sup>1</sup>	Plant growth promotion in vitro	Plant growth promotion (greenhouse)	References
<i>Kosakonia radicincitans</i> UYSO10	Sugarcane (cv. FAM81-72), stems	BNF, IAA	Sugarcane cv. LCP85-384	Sugarcane cv. LCP85-384	Taulé et al. 2012; 2016; 2019
<i>Shinella</i> sp. UYSO24	Sugarcane (cv. TUC77-42), stems	IAA	Sugarcane cvs. LCP85-384	Sugarcane cv. LCP85-384	Taulé et al. 2012; 2016
<i>Rhizobium</i> sp. UYSB13	Sweet sorghum (cv. M81E), roots	ACC, <i>nifH</i> <sup>+</sup>	Sweet sorghum cv. M81E.	ND	Mareque et al. 2015
<i>Pantoea</i> sp. UYSB45	Sweet sorghum (cv. M81E), roots	IAA, Sid <sup>+</sup> , P solubilization	Sweet sorghum cv. M81E.	ND	Mareque et al. 2015
<i>Enterobacter</i> sp. UYSB50	Sweet sorghum (cv. M81E), roots	<i>nifH</i> <sup>+</sup> , IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Enterobacter</i> sp. UYSB68	Sweet sorghum (cv. M81E), roots	<i>nifH</i> <sup>+</sup> , IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Enterobacter</i> sp. UYSB73	Sweet sorghum (cv. M81E), roots	BNF, IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Kosakonia</i> sp. UYSB88	Sweet sorghum (cv. M81E), roots	<i>nifH</i> <sup>+</sup> , IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Enterobacter</i> sp. UYSB89	Sweet sorghum (cv. M81E), roots	BNF, IAA	Sweet sorghum cv. M81E.	Sweet sorghum cv. M81E, ADV2010.	Heijo et al. 2021
<i>Acinetobacter</i> sp. UYSB93	Sweet sorghum (cv. M81E), roots	BNF, IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Kosakonia</i> sp. UYSB106	Sweet sorghum (cv. M81E), roots	<i>nifH</i> <sup>+</sup> , P solubilization	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Bacillus</i> sp. UYSB119	Sweet sorghum (cv. M81E), root	BNF, IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Kosakonia</i> sp. UYSB139	Sweet sorghum (cv. M81E), seeds	<i>nifH</i> <sup>+</sup> , IAA	Sweet sorghum cv. M81E.	Sweet sorghum cv. M81E, ADV2010.	Heijo et al. 2021
<i>Kosakonia</i> sp. UYSB 152	Sweet sorghum (cv. M81E), stems	<i>nifH</i> <sup>+</sup> , IAA	Sweet sorghum cv. M81E.	ND	Heijo et al. 2021
<i>Herbaspirillum huttei</i> strain 4.2	Rice ( <i>Oryza sativa</i> , INIA Olimar), stems and leaves	BNF	Rice cv, El Paso 144	Rice cv. El Paso 144 <sup>2</sup>	Punschke and Mayans 2011
<i>Streptomyces albus</i> UYFA156	Tall fescue (cv. SFRO Don Tomás), seeds	-	Tall fescue cv. SFRO Don Tomás	Tall fescue cv. SFRO Don Tomás	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Microbacterium</i> sp. UYFA68	Tall fescue (cv. SFRO Don Tomás), roots	IAA	Tall fescue cv. SFRO Don Tomás	Tall fescue cv. SFRO Don Tomás and Tacuabé	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Microbacterium</i> sp. UYFA09	Tall fescue (cv. SFRO Don Tomás), stems	<i>nifH</i> <sup>+</sup>	Tall fescue cv. SFRO Don Tomás	Tall fescue cv. SFRO Don Tomás	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Pantoea</i> sp. UYFA15	Tall fescue (cv. SFRO Don Tomás), stems	<i>nifH</i> <sup>+</sup>	Tall fescue cv. SFRO Don Tomás	Tall fescue cv. Tacuabé	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Variovorax</i> sp. UYFA22	Tall fescue (cv. SFRO Don Tomás), stems	<i>nifH</i> <sup>+</sup>	Tall fescue cv. SFRO Don Tomás,	-	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Microbacterium</i> sp. UYFA26	Tall fescue (cv. SFRO Don Tomás), stems	IAA	Tall fescue cv. SFRO Don Tomás	-	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Microbacterium</i> sp. UYFA61	Tall fescue (cv. SFRO Don Tomás), roots	IAA	Tall fescue cv. SFRO Don Tomás	Tall fescue (cv. SFRO Don Tomás)	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Curtobacterium</i> sp. UYFA157	Tall fescue (cv. SFRO Don Tomás), seeds	<i>nifH</i> <sup>+</sup>	Tall fescue cv. SFRO Don Tomás	Tall fescue (cv. SFRO Don Tomás)	De los Santos et al. 2015; Vaz-Jauri et al. 2019.

**Table 1** (continued)

Strain	Plant source	Plant growth promotion mechanism (in vitro) <sup>1</sup>	Plant growth promotion in vitro	Plant growth promotion (greenhouse)	References
<i>Pseudomonas</i> sp. UYFA249	Tall fescue (cv. SFRO Don Tomás), roots	IAA	Tall fescue cv. SFRO Don Tomás	Tall fescue (cv. SFRO Don Tomás and Tacuabé)	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Xhantomonas</i> sp. UYFA215	Tall fescue (cv. SFRO Don Tomás), roots	<i>nifH</i> <sup>+</sup>	Tall fescue cv. SFRO Don Tomás	-	De los Santos et al. 2015; Vaz-Jauri et al. 2019.
<i>Pseudomonas</i> sp. UYBN151	Canola (cv. Igranola 103), roots	IAA, Sid <sup>+</sup> , P solubilization, <i>nifH</i> <sup>+</sup>	Canola cv. Igranola103	ND	Ferrari et al. 2022
<i>Arthrobacter</i> sp. UYBN204	Canola (cv. Igranola 103), stems	<i>nifH</i> <sup>+</sup> , IAA	Canola cv. Igranola103	ND	Ferrari et al. 2022
<i>Pseudomonas fluorescens</i> EMA68	Maize (Tendem), steem	Sid <sup>+</sup> , P solubilization, IAA	Maize cv. NK900		Montañez et al. 2009, 2012
<i>Pantoea</i> sp. EMA35	Maize (PAU785), stem	P solubilization, IAA	Maize cv. DK682		Montañez et al. 2009, 2012
<i>Pantoea agglomerans</i> EMA82	Maize (Topacio), stem	P solubilization, IAA, BNF		Maize cv. PAU871	Montañez and Sicardi 2013)
<i>Enterobacter</i> sp. EMA130	Maize (NK900), stem	P solubilization, IAA	Maize cv. DK682		Montañez et al. 2009, 2012
<i>Enterobacter</i> sp. EMA15	Maize (DK688), root	P solubilization, IAA, BNF	Maize cv. NK900		Montañez et al. 2009, 2012
<i>Enterobacter</i> sp. EMA149	Maize (Cheyenne), stem	P solubilization, IAA	Maize cv. NK900		Montañez et al. 2009, 2012
<i>Pseudomonas fluorescens</i> EMA38	Maize (PAU785), stem	P solubilization, IAA	Maize cv. NK900		Montañez et al. 2009, 2012
<i>Herbaspirillum frisingense</i> EMA117	Maize (NK940), stem	P solubilization, IAA, BNF	Maize cv. NK900	Maize cv. PAU871	Montañez et al. 2009, 2012. Montañez & Sicardi, 2013
<i>Rhanelia</i> sp. EMA83	Maize (Topacio). stem	P solubilization, IAA, BNF		Maize cvs. PAU871, Maizon, NK900 <sup>2</sup>	Montañez and Sicardi 2013

<sup>1</sup>BNF: biological nitrogen fixation capacity demonstrated by Acetylene Reduction Assay, IAA: indole acetic acid production, Sid: siderophore production, *nifH*<sup>+</sup>: *nifH* gene detected by PCR approach. <sup>2</sup>PGP confirmed under field experiments

the PGP strain *Streptomyces albus* UYFA156 was further characterized, defined as a true endophyte of both cultivars evaluated, and its genome was sequenced (Vaz Jauri et al. 2019a, b). Notable differences in the response of these two cultivars to inoculation with the tested strains led to further research, including the study of the endophytic bacterial community of the two cultivars by sequencing the *16S rDNA* gene (Vaz Jauri et al. 2022). The results revealed significant differences in identities at all taxonomic levels tested (phylum, order, family, and genus) within each community of the two cultivars evaluated. For example, although bacteria belonging to the phylum Proteobacteria were overwhelmingly more abundant in all treatments, the proportion of this group in the Ta cultivar was significantly higher than that in the DT cultivar. However, DT bacterial endophytic communities were richer in Firmicutes and Synergistetes. In addition, the response of these communities to inoculation with an endophyte plant growth-promoting

strain, *Streptomyces albidoflavus* UYFA156, obtained from the collection mentioned above, also varied between the growth promotion-responsive and -unresponsive cultivars. Specifically, diversity increased significantly within days in the natural host but not in the other tall fescue cultivar.

### Soybean (*Glycine max*)

Soybean is a legume crop cultivated worldwide and is used in diverse ways for human and animal consumption. In Uruguay, soybean is the most important crop and in 2021 it covered 56% of the total cultivated area (MGAP/DIEA, 2022). For a comprehensive and up-to-date review on soybean-rhizobia interaction please refer to Fabiano et al. (2023). Here we will focus on mycorrhizal-soybean interaction. In this sense, the effect of different rotations in soybean crops on mycorrhizal colonization, soil glomalin content, and AMF diversity was analyzed in a long-term agricultural rotation

experiment using the T-RFLP technique (Rodríguez-Blanco and Giménez 2019). This work was based on two rice-soybean contrasting rotations: one with intensive use, which includes 4 years of crops, and one with less intensive use, including rice-soybean-2 years of pasture. The results showed that the roots of soybean plants were highly colonized by diverse communities of AMF, even under intensive soil use. The diversity and mycorrhizal colonization of soybean were affected by the identity of the previous crop. Specifically, the two parameters measured were lower when the previous crop was rice instead of soybean. Rice roots were poorly colonized by AMF in waterlogged paddy fields and the authors suggested this could partially explain the results. Furthermore, continuous cropping of soybean can lead to changes in soil nutrients, microorganisms, and enzyme activities that could be beneficial for AMF, as it was postulated by Wang et al. (2012). In addition, the glomalin content did not differ between the different rotations. These results referred to the initial stage of the long-term experiment, so authors postulate that it will be necessary to continue assessing mid- and long-term variations in AMF communities.

### Rice (*Oryza sativa*)

Rice belongs to the Poaceae family and is the most important cereal crop, feeding more than 50% of the world's population (Mano and Morisaki 2008). In Uruguay, rice cropping represented 7% in 2021 of the total cultivated area (MGAP/DIEA, 2022) and is characterized by intermittent drainage and rotation with pastures, employing minimal amounts of agrochemicals, particularly nitrogen ( $45\text{--}70\text{ kg of N ha}^{-1}$ ), compared with other rice systems in the world (Deambrosi 2003). The intensification of crop production has allowed for impressive yield increases in recent decades (~60% increase in national production) (Pittelkow et al. 2016), although the intensification accompanies the increase in N fertilization and causes concerns for sustainability due to the risk of N loss to the environment (Tseng et al. 2021). Therefore, plant-associated bacteria with the ability to fix  $\text{N}_2$  may be relevant for the sustainable increase in rice production. The question of whether there is a core microbiota associated with different rice cultivars was studied in the following three rice varieties: INIA Tacuarí (type Japónica), INIA Olimar, and El Paso 144 (type Índica). The diversity of endophytic bacterial communities associated with leaves of these three rice varieties was evaluated in two crop seasons by culture-based techniques and 16S rRNA T-RFLP analysis (Ferrando et al. 2012). The endophytic communities were composed of a reduced group of strongly associated and persistent bacteria that were partially recovered by cultivation (Fig. 1). While *Gammaproteobacteria*

dominated culture-retrieved leaf endophytes (mainly *Pantoea ananatis* and *Pseudomonas syringae*), *Alphaproteobacteria* (mainly *Brevundimonas*) dominated rice leaf endophytic communities according to 16S rRNA gene sequences. Different rice cultivars harbored microbial communities that were highly similar, although a certain degree of host specificity by rice cultivar was observed. Additionally, a 16S rRNA gene amplicon sequencing analysis performed on a long-term rice field experiment showed that the crop growth stage and the niche (roots, bulk and rhizospheric soils) were the main drivers of the structure and composition of rice microbiomes (Martínez-Pereyra 2020). Moreover, root-associated microbiomes were more sensitive to changes in crop stage (which implies flooded conditions) than bulk soil microbiomes. The microbiota associated with rice roots (rhizospheric and endophytic) detected by molecular methods resulted in a functionally diverse community capable of adapting to changing conditions and harboring great potential for carrying out relevant processes to agriculture and the environment, such as nitrogen fixation, nitrous oxide reduction, methane oxidation, sulfate reduction and fermentation and decomposition of plant tissues (Martínez-Pereyra 2020). The diversity of diazotrophic root endophytic communities was studied in plants from a greenhouse experiment (Ferrando and Fernández Scavino 2015). Cloning and 454 pyrosequencing of the *nifH* gene revealed that, in dry soil (at the tillering stage), root endophytes were dominated by the gammaproteobacterial genus *Stenotrophomonas*. A physiologically and metabolically diverse community dominated by facultative and strict anaerobes was established inside rice roots after flooding. Another *nifH* gene-based diversity study (T-RFLP) in plants from a field experiment showed that diazotrophic communities from soil and from rice roots were different and confirmed that more diverse diazotrophic communities were established at the flooding stage (Ghiazza 2018). Culture-based techniques allowed the detection of diazotrophs associated with rice roots belonging to diverse physiological guilds, such as anaerobic heterotrophs and sulfate reducers (Ghiazza 2018) or aerobic methane-oxidizing bacteria affiliated with the genera *Methylosinus* and *Methylocystis* (Ferrando and Fernández Scavino 2015). These retrieved bacterial guilds participate directly or indirectly in biogeochemical processes involved in methane emission under flooded conditions, with rice paddies being a major source of this potent greenhouse gas (Kögel-Knabner et al. 2010). Additionally, plant-associated bacteria with different plant growth-promoting properties were isolated from rice. Bacteria belonging to the genera *Azospirillum*, *Herbaspirillum*, *Enterobacter*, *Paenibacillus* and *Pleomorphomonas* able to fix  $\text{N}_2$  were isolated from roots and aerial parts of the plant, whereas phosphate-solubilizing bacteria of the genus

*Burkholderia* were isolated from the rice rhizosphere (Fig. 1) (Rariz et al. 2013). Remarkably, these isolates exhibited additional PGP properties, such as siderophore or IAA production. In particular, siderophore-producing bacteria (SPB) were present in grains, roots, and leaves of rice, and their relative proportion among heterotrophic bacteria increased in mature plants (Loaces et al. 2010). Within the diverse community of SPB, the genus *Pantoea* was dominant, with *Pantoea ananatis* being the main species permanently associated with roots and leaves (Fig. 1). In addition, among the strongest SPB, bacteria of the genus *Burkholderia* (Fig. 1) with powerful antagonistic activity against the phytopathogenic fungi *Rhizoctonia oryzae* and *Sclerotium oryzae* were isolated mainly from flooded rice roots (Loaces et al. 2010). Diverse genera or bacterial groups have been explored to search for potential PGP properties to apply for rice cropping in Uruguay. Punschke and Mayans (2011) retrieved an endophytic diazotrophic bacterium from rice leaves, strain 4.2 of the genus *Herbaspirillum* (Table 1), which increased the biomass of roots and aerial parts of the plant in greenhouse experiments. In field experiments, the inoculation with this strain augmented the yield by 15% compared with that of non-inoculated plants with reduced N fertilization (Punschke and Mayans 2011). The first rice-specific biofertilizer produced and registered in Uruguay was developed with this strain (MGAP, 2022). On the other side, cyanobacteria may be relevant for the fertility of rice paddy soils. The highest density of cyanobacteria in Uruguayan rice fields occurred two months after flooding when heterocystous filamentous cyanobacteria belonging to the genera *Nostoc* and *Anabaena* were observed (Irisarri et al. 2001). Strains of the genera *Nostoc* and *Calothrix*, isolated from Uruguayan rice paddy soils, exhibited promising properties for plant growth promotion, such as high growth rates and photosynthetic and nitrogenase activities, but exhibited a low capacity to release  $\text{NH}_4^+$  directly into the environment (Pérez et al. 2020). However, the inoculation with native cyanobacterial isolates (*Nostoc* BI42, *Anabaena* BI46, and *Calothrix* BI22) did not affect the grain yield in field experiments (Irisarri et al. 2006). The diversity, persistence, and dynamics of rice-associated bacteria may have practical implications. The interactions between native microbiota and PGPB from commercial inoculants may be relevant for the success of the bioinoculant as well as for the preservation of the source of new potential PGPB. Therefore, *Pseudomonas oryzihabitans*, seed-borne rice endophyte-producing siderophores, was able to antagonize *Azospirillum brasilense* Az39, a PGPB widely used in South America for grasses (Rariz et al. 2017). The strain G16 of *P. oryzihabitans* overgrew Az39 and reduced the seedling biomass when both strains were co-inoculated at similar densities in disinfected seeds. Thus, conditions that favor the growth of certain endophytic native

populations may facilitate PGPB colonization. On the other hand, it was observed that after planting in soil, disinfected seeds were rapidly colonized by diazotrophic bacteria. Moreover, when these seeds were previously inoculated with *A. brasilense* Az39 or *Herbaspirillum seropedicae* Z67 (two PGPB present in bioinoculants commercialized in the region for grasses), the diversity of native diazotrophs associated with rice roots was reduced, according to *nifH* gene sequence analyses (Rariz et al. 2017). Therefore, native rice-associated microbiota offers a wide range of potentially beneficial plant bacteria with several relevant properties not only for the plant but also for environmental biogeochemical processes. Some of these properties may make the resident microbiota successful in competition with PGPB applied in commercial bioinoculants and reduce its efficiency. In addition, it is also intriguing to know whether the systematic use of bioinoculants threatens the diversity of one of the main sources of potential PGPB, namely, plant-associated bacteria.

### Sugarcane (*Saccharum officinarum*)

Sugarcane belongs to the Poaceae family and is considered a multipurpose crop used in Uruguay for energy, feed, sugar, and bioethanol production, although it represents a small proportion of the cultivated area (0.3% in 2021, MGAP/DIEA 2022). This crop demands high doses of N-chemical fertilization for optimal growth. In this context, several investigations were conducted with the aim of studying the endophytic diazotrophic community associated with sugarcane cultivars grown in Uruguay (CP 92–618, LCP 85–384, TUC 77–42, TUC 78–12, TUC 67–27 and FAM 81–77) as a starting point for bioinoculant development. First, by using the  $^{15}\text{N}$ -dilution technique, it was shown that Uruguayan sugarcane cultivars obtain significant inputs of N (34.8–58.8%) from the BNF process. Later, from those cultivars, a collection of 598 bacteria associated with the internal stem tissues was constructed, and those potential diazotrophic isolates were subjected to further characterization. Plant growth-promoting features were detected in 35 selected isolates, including the capacity to solubilize P and Fe and produce IAA, demonstrating a broad range of metabolic features for potential biotechnology applications. Additionally, part of the collection was identified through 16S sequencing, and the isolates were shown to belong to the genera *Pseudomonas*, *Stenotrophomonas*, *Xanthomonas*, *Acinetobacter*, *Rhanella*, *Enterobacter*, *Pantoea*, *Shinella*, *Agrobacterium* and *Achromobacter* (Fig. 1) (Taulé et al. 2011). In addition, the PGP potential of some of these isolates was evaluated in vitro and greenhouse assays. From these experiments, the strains *Kosakonia* sp. UYSO10 and *Shinella* sp. UYSO24 were identified as PGP of sugarcane

micropropagated plants and cuttings of the more extensively planted cultivar LCP 85,384 (Table 1) (Taulé et al. 2016, 2019). To deeply characterize the interaction of these isolates and sugarcane plants, the colonization and infection processes were studied and described using a microscopic and molecular biology approach. The results show that both strains are true endophytes of the sugarcane plant cultivar LCP 85,384 (Taulé et al. 2016). Moreover, the endophytic PGP strain UYSO10 was further studied; its genome was sequenced and identified at the species level as *K. radicincitans* by phylogenetic genomic studies (Beracochea et al. 2019). In addition, *in silico* analyses of the UYSO10 genome revealed the presence of two clusters encoding nitrogenase enzymes, MoFe-nitrogenase and FeFe-nitrogenase (Taulé et al. 2011; Beracochea et al. 2019). Single and double mutants in the nitrogenases structural genes (*nifH* and *anfH*) were constructed, and their characterization indicated that both nitrogenases are functional and necessary for the plant growth promotion of the sugarcane cultivar LCP 85,384 (Taulé et al. 2019). Taken together, the results demonstrated a diversity of bacterial endophytes associated with sugarcane cultivars cultivated in Uruguay, with a high biotechnology potential. Moreover, some of these isolates were PGPM of common cultivars used in Uruguay, and in one of them, BNF was identified as one of the mechanisms involved.

### Sweet sorghum (*Sorghum bicolor*)

Sweet sorghum belongs to the Poaceae family and, globally, is the fourth most important cereal, which is known as a multipurpose crop, and is used as a feedstock to produce bioethanol, sugar, energy, and animal feed. In Uruguay in 2021, it represented 0.9% of the total cultivated area of the country (MGAP/DIEA 2022). However, high levels of N-chemical fertilization are required to achieve optimal growth, causing environmental degradation. With the aim of understanding how these practices affect the bacterial microbiome associated with field-grown sweet sorghum, a culture-independent approach was used to evaluate the effects of N-chemical fertilization on the structure, diversity, abundance, and composition of the endophytic bacterial microbiome. For PCR-DGGE, quantitative PCR and high-throughput sequencing were performed based on the amplification of the 16S rRNA and *nifH* genes (Mareque et al. 2018). The results showed that the level of N fertilization affected the structure, composition, and abundance but not the diversity of the endophytic bacterial communities associated with the sweet sorghum plant cultivar M81E. This effect was pronounced in the roots of both bacterial communities analyzed and may depend on the physiological state of the plants. In addition, and as a starting point for

bioinoculant development, a collection of 369 endophytic bacteria associated with cultivar M81E was constructed from surface-disinfected stems, roots, and seeds. The collection was genetically and biochemically characterized, with a focus on PGP and infection traits, and a subset of these isolates was identified (Mareque et al. 2015; Heijo et al. 2021). Bacterial isolates were shown to belong to different genera, including *Pantoea*, *Enterobacter*, *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, *Ralstonia*, *Herbaspirillum*, *Achromobacter*, *Rhizobium*, *Chryseobacterium*, *Kocuria*, *Brevibacillus*, *Paenibacillus*, *Bacillus*, *Staphylococcus*, *Klebsiella*, *Kosakonia*, *Citrobacter*, *Cellulomonas* and *Microbacterium*, demonstrating high diversity (Fig. 1) (Mareque et al. 2015; Heijo et al. 2021). To evaluate the potential to promote sweet sorghum growth, a subset of these isolates was inoculated under *in vitro* and greenhouse conditions. From these assays, the strains *Enterobacter* sp. UYSB89 and *Kosakonia* sp. UYSB139 were shown to promote the growth of cultivars M81E and ADV2010 (Table 1). Further assays were carried out to analyze the plant response to the combination of N fertilization and bacterial inoculation. The results indicate that the evaluated cultivars responded differentially to bacterial inoculation, nitrogen fertilization doses, and the interaction of both factors (Heijo et al. 2021). These observations highlight the fact that plant growth is a multifactorial consequence of the interrelationship between crop practices and plant genotypes. Moreover, the results suggest that a reduction in chemical nitrogen fertilization in the field should be evaluated.

### Maize (*Zea mays* L.)

Maize is an important multipurpose crop that belongs to the Poaceae family, and its production has been increasing significantly during the last 10 years in the southern Latin American countries Argentina, Brazil, Paraguay and Uruguay (Ranum et al. 2014). In Uruguay, maize-planted area represented 6.9% of the total cultivated area of the country in 2021 (MGAP/DIEA 2022). Maize crop production requires an improvement in its environmental sustainability since its optimal growth also depends on the application of high amounts of N-chemical fertilization. As a starting point for bioinoculant development based on PGP endophytic bacteria, several investigations were conducted. First, using the <sup>15</sup>N-dilution technique, it was demonstrated that the cultivars (DK-682, DK-688, Tendem, Topacio, Condor, Suco, NK-940, NK-900, Maizon, and PAU-871) grown in Uruguay obtained significant inputs of N (12–33%) from the BNF process (Montañez et al. 2009).

In addition, culturable bacterial endophytes were isolated from the same cultivars mentioned and showed a high diversity of associated bacteria, including the genera *Rhanelia*,



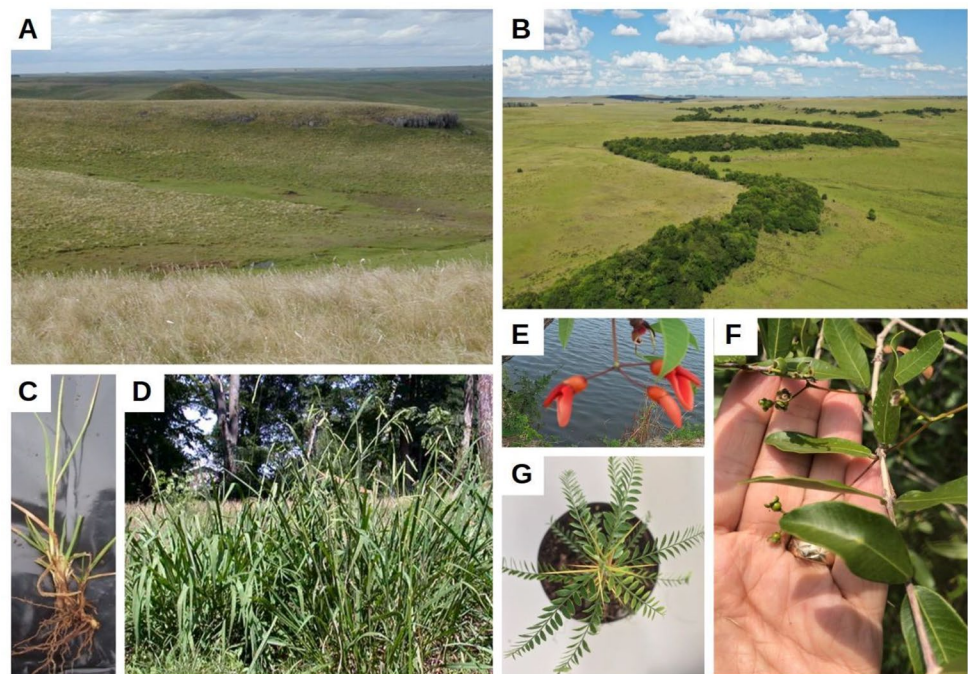
*Pantoea*, *Rhizobium*, *Pseudomona*, *Herbaspirillum*, *Enterobacter*, *Brevundimonas* and *Burkholderia* (Fig. 1). The effect of the genotype and N fertilization on the community associated with cultivars NK940 and PAU871 was studied. The results showed that the maize genotype had a marked effect on the number and diversity of endophytic communities, particularly the cultivar NK940 community being more abundant and diverse than that of the cultivar PAU871. Moreover, N fertilization increased the number and diversity of diazotrophs in endophytic communities but not in rhizosphere samples (Rodríguez-Blanco et al. 2015). Furthermore, the plant growth promotion of maize cultivars NK900 and NK682 by bacterial inoculation was evaluated under in vitro conditions. PGP isolates related to the genera *Rhanella*, *Enterobacter*, *Pseudomona*, *Pantoea*, *Brevundimonas*, *Rhizobium* and *Herbaspirillum* were identified (Montañez et al. 2012). The PGPB identified in vitro were evaluated as inoculants in greenhouse conditions (Table 1). In the greenhouse experiment mentioned, the  $^{15}\text{N}$  isotope dilution method was used to estimate the BNF among maize cultivars (Maizón, PAU871, and NK940) inoculated and non-inoculated. In general, an increase in shoot dry weight was observed among inoculated plants with higher N content. In particular, the results showed significant differences in shoot and root dry weight among cultivars and inoculation treatments in the greenhouse. Furthermore, it was observed that a *Rhanella* sp. isolate increased shoot biomass upon inoculation of the Maizón and PAU871 cultivars, while *Pantoea* sp. isolate increased biomass only in the cultivar PAU871. Grounded on previous greenhouse studies, the effects of strain *Rhanella* sp. EMA83 inoculation on cultivar

NK900 was evaluated in a field experiment, demonstrating a PGP capacity (Montañez and Sicardi 2013). Taken together, the results highlight the potential of endophytic bacteria associated with maize for bioinoculant development.

## Non-cultivated species

Natural grasslands are the main ecosystem of Uruguay; it covers 60% of the total country area and in the last two decades it has been reduced by over 10% (Proyecto Map-Biomas 2023, Fig. 2). Recent studies have focused on the ecology of the interaction between arbuscular mycorrhizal fungi (AMF) and native grasses of grasslands, (Parodi and Pezzani 2011; García et al. 2016, 2017, 2019). More recently, the effect of phosphorus (P) fertilization on the diversity of endomycorrhizae associated with two native grasses that are very frequent and abundant in Uruguayan grasslands (*Paspalum dilatatum* and *Coelorhachis selloana*, Fig. 2) was analyzed (García et al. 2017). The diversity and abundance of AMF spores in the rhizosphere of these grasses were studied using morphological techniques, while the diversity of AMF colonizing root systems was studied using T-RFLP. The increase in available P concentration in the soil did not affect the diversity of arbuscular mycorrhizae, either in the rhizosphere or within roots. There was an effect of the host species on the richness of AMF biotypes in the roots and on the abundance of spores in the soil. *C. selloana* presented higher values in relation to *P. dilatatum*. Furthermore, differences were found in the diversity of AMF depending on the season of the year, with the number

**Fig. 2** Native landscapes and studied species. **A:** Uruguayan prairie (photo Ecología de Pastizales group); **B:** riparian forest, Salto, Uruguay (photo: Biodiversidad y Ecología de la Conservación group); **C:** Uruguayan native grass *Coelorhachis selloana* (photo: Gerardo Parodi); **D:** Uruguayan native grass *Paspalum dilatatum* (photo: Silvina García); **E:** riparian forest native tree, *Erythrina crista-galli* (photo: Patricia Vaz Jauri); **F:** riparian forest native tree *Eugenia uruguayensis* (photo: Christine Lucas); **G:** riparian forest native tree *Gleditsia amorphoides* (photo: Sofia Acosta Nabune)



of AMF biotypes being greater in winter than in summer. Mycorrhizas in Uruguay were scarcely studied in woody plant species (Malvárez et al. 1997). Frioni et al. (1999) analyzed the roots of 23 native species of tree and shrub legumes to explore the presence of ecto- and endomycorrhizae, as well as the density and diversity of AMF, through a morphological group classification of AMF spores. All the legume species studied presented endomycorrhizae, while only 26% of the species showed colonization by ectomycorrhizae. AMF spore density was high in all soils analyzed, and the dominant spore morphotypes were classified into at least three morphological groups. Considering the ubiquity of mycorrhizae in plant root systems, both in natural ecosystems and agroecosystems, and their potential application as plant growth promoters, it is necessary to increase knowledge of the diversity of native mycorrhizal-forming fungi in Uruguay and analyze the effects of different management practices on them.

### Wild trees of riparian forests

Riparian forests, which occur along streams and rivers, are the largest type of forest in Uruguay, spanning a total area of 743.249 ha (4.2% of the country, Fig. 2) (MGAP – MVOTMA, 2019). They provide ecosystem functions that are important for biodiversity maintenance in both terrestrial and aquatic ecosystems, playing a fundamental role in the protection of water quality through sediment filtration, conservation of habitat structure, temperature regulation and carbon sequestration (Chazdon 2014). This ecosystem is threatened by the increase in commercial forests and agricultural-livestock pressure, along with the invasion of exotic species. In natural forests, seeds and seedlings are the stages in which the highest mortality rates occur, becoming the most important bottlenecks in the life cycle of trees (Petit and Hampe 2006; Leck et al. 2008; Bever et al. 2015). Thus, the implementation of the active restoration of forests by planting trees requires strong and healthy plants, such that their performance (survival and growth) will enable adequate ecosystem recovery. With the aim of future contributions to restoration programs, a collection of endophytic bacterial isolates from seeds of wild trees growing in riparian forests of the lower Uruguay River was created (Fig. 1) (Vaz Jauri et al., this issue). The seeds were obtained from trees that belong to the native species *Erythrina crista-galli* and *Gleditsia amorphoides* of the family Fabaceae, *Myrcianthes cisplatensis* and *Eugenia uniflora* of the family Myrtaceae, and *Nectandra angustifolia* of the family Lauraceae. In addition, seeds of the exotic and highly invasive species *Gleditsia triacanthos*, also a Fabaceae, were studied (Fig. 2). Bacteria were characterized both functionally

and taxonomically. Plant growth-promoting activity was evaluated in vitro in a model system (*Brassica juncea*), and a selected isolate from *G. amorphoides* was validated as a growth promoter in *E. crista-galli*. Although this line of work is in its initial stages, it has a high potential to contribute both to biotechnology and to the understanding of microbial ecology in natural ecosystems.

### Concluding remarks

The starting point for the development of bioinoculants requires the construction and characterization (in vitro and in vivo) of collections of microorganisms associated with plants and crops of interest; as well as understanding the microbial ecology that prevails in these type of interactions. In this sense, The studies carried out in Uruguay on the rhizospheric and endophytic microbiota focused on cultivated and non-cultivated plant species. The results showed a great diversity of microorganisms with different PGP traits associated with the plants under study, stressing their biotechnological potential. Furthermore, a bioinoculant for rice based on a strain of the genus *Herbaspirillum* was developed and commercialized. Taking into account the fact that the PGP capacity of several isolates has already been confirmed under greenhouse conditions, it would be interesting to evaluate them under field conditions to corroborate the PGP effects for further biotechnological development. During this process, one must not forget to carry out the different types of quality control on the strains (especially those related to pathogenicity), following the existing regulations in the country where the bioinput is to be commercialized and applied.

### Declarations

**Competing interests** The authors declare that they have no competing interests. We confirm that all authors were agreed to submit the paper in Journal of Environmental Sustainability.

**Ethics approval and consent to participate** The works present in this paper is an original and carried out solely by us. We confirm that the manuscript does not contain experiments using animals nor human studies.

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