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Analyzing the Hemp Microbiota: Isolation and Characterization of Endophytic Bacteria Associated to *Cannabis sativa* cv. Luma as a First Step towards Bioinput Development

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Abstract

Plants are naturally associated with microorganisms' communities (microbiotas), which have a significant influence on the plant phenotype, extending its genetic potential and conferring essential traits for growth, resilience, and health. Within these microbiotas, bacterial endophytes are of particular relevance due to their widely distributed plant growth-promoting (PGP) activities and their capacity to synthesize secondary metabolites. These features make them promising candidates for biotechnological applications, particularly the development of bioinputs (biofertilizers, biostimulants, and biopesticides), aimed at reducing dependence on chemical inputs in agriculture. Current hemp (*Cannabis sativa*) cultivation practices rely heavily on chemical inputs, generating economic, environmental, and social challenges. This study reports the construction and characterization of a collection of endophytic bacteria associated with healthy plants and seeds of the hemp cultivar Luma, a crop cultivated in Uruguay primarily for fiber production. A total of 236 bacterial isolates were obtained from surface-disinfected seeds, seedlings, roots, and aerial tissues, confirming their status as putative endophytes. Of these, 66% of the isolates exhibited at least one of the 5 PGP traits tested, including nitrogen fixation, mineral solubilization, and phytohormone production. Genera with recognized PGP potential, such as *Bacillus* and *Pseudomonas*, were particularly abundant in seeds and roots, respectively. Notably, this is the first report describing the association of *Mycolicibacterium* and *Aminobacter* strains with cannabis plants. This work represents a first step toward developing bioinputs tailored to the Uruguayan cannabis industry and highlights the broader promise of endophytes for sustainable agricultural practices.

Keywords: cannabis, microbiota, health, plant growth promotion, plant protection

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Analizando la microbiota del cáñamo: Aislamiento y caracterización de bacterias endófitas asociadas a *Cannabis sativa* cv. Luma como primer paso hacia el desarrollo de bioinsumos

Resumen

Las plantas se asocian de forma natural con comunidades de microorganismos (microbiotas) que influyen en su fenotipo, lo que amplía su potencial genético y les otorga rasgos claves para su crecimiento, salud y resiliencia. Entre estas comunidades, los endófitos bacterianos destacan por sus actividades de promoción del crecimiento vegetal (PCV) y su capacidad para sintetizar metabolitos secundarios, lo que los posiciona como candidatos idóneos para desarrollar bioinsumos (biofertilizantes, bioestimulantes y bioplaguicidas) que reduzcan el uso de agroquímicos. En Uruguay, el cultivo de cáñamo (*Cannabis sativa*) depende fuertemente de insumos químicos, lo que genera problemas económicos, ambientales y sociales. Este estudio reporta la construcción y la caracterización de una colección de bacterias endófitas asociadas a plantas sanas y semillas del cultivar Luma, producido en Uruguay principalmente para fibra. Se construyó una colección con 236 aislamientos bacterianos a partir de semillas, plántulas, raíces y tejidos aéreos desinfectados superficialmente, confirmando su condición de endófitos. De esta colección, 66 % de los aislamientos presentó al menos una de las cinco actividades PCV evaluadas, tales como fijación de nitrógeno, solubilización de minerales y producción de fitohormonas. Géneros reconocidos con potencial PCV, como *Bacillus* y *Pseudomonas*, abundaron en semillas y raíces, respectivamente. Cabe destacar que este es el primer informe que describe la asociación de cepas de *Mycolicibacterium* y *Aminobacter* con plantas de cannabis. Este trabajo representa un primer paso hacia el desarrollo de bioinsumos adaptados a la industria del cannabis en Uruguay y resalta el promisorio papel de los endófitos para prácticas agrícolas sostenibles.

Palabras clave: cannabis, microbiota, salud, promoción del crecimiento vegetal, protección de plantas

Analisando a microbiota do cânhamo: Isolamento e caracterização de bactérias endofíticas associadas à *Cannabis sativa* cv. Luma como primeiro passo para o desenvolvimento de bioinsumos

Resumo

As plantas estão naturalmente associadas a comunidades de microrganismos (microbiotas), que exercem influência significativa sobre o fenótipo vegetal, ampliando seu potencial genético e conferindo características essenciais para o crescimento, resiliência e saúde. Dentro dessas microbiotas, os endófitos bacterianos são de particular relevância devido às suas amplamente distribuídas atividades de promoção do crescimento vegetal (PGP) e à capacidade de sintetizar metabólitos secundários. Essas características os tornam candidatos promissores para aplicações biotecnológicas, especialmente no desenvolvimento de bioinsumos (biofertilizantes, bioestimulantes e biopesticidas), visando reduzir a dependência de insumos químicos na agricultura. As práticas atuais de cultivo de cânhamo (*Cannabis sativa)* dependem fortemente de insumos químicos, gerando desafios econômicos, ambientais e sociais. Este estudo relata a construção e caracterização de uma coleção de bactérias endófitas associadas a plantas saudáveis e sementes da cultivar de cânhamo Luma, uma cultura cultivada no Uruguai principalmente para produção de fibra. Foram obtidos um total de 236 isolados bacterianos a partir de sementes, plântulas, raízes e tecidos aéreos desinfetados superficialmente, confirmando seu status como endófitos putativos. Desses, 66% dos isolados apresentaram pelo menos uma das 5 atividades PGP testadas, incluindo fixação de nitrogênio, solubilização de minerais e produção de fitormônios. Gêneros com potencial PGP reconhecido, como *Bacillus* e *Pseudomonas*, foram particularmente abundantes em sementes e raízes, respectivamente. Vale destacar que este é o primeiro relato descrevendo a associação de cepas de *Mycolicibacterium* e *Aminobacter* com plantas de cannabis. Este trabalho representa um primeiro passo para o desenvolvimento de bioinsumos adaptados à indústria do cannabis no Uruguai e destaca o promissor papel dos endófitos para práticas agrícolas sustentáveis.

Palavras-chave: cannabis, microbiota, saúde, promoção do crescimento vegetal, proteção de plantas

1. Introduction

Plants and their associated microbiotas are collectively known as the plant holobiont (Lyu et al., 2021; Rosenberg & Zilber-Rosenberg, 2016). The holobiont consists of the plant and all the microorganisms (protozoa, fungi, bacteria, viruses) that live on or within the plant (Berg et al., 2014). As a result, the final phenotype of the plant is shaped by the interactions among all holobiont components. In this context, the plant-associated microbiotas extend the genetic potential of the plant, conferring unique characteristics (Berg et al., 2014; Johnston-Monje et al., 2023; Vandenkoornhuyse et al., 2015). Among these microbiotas, the bacterial endophytic microbiota comprising bacterial communities that reside within the plant internal tissues plays a critical role in the plant health, growth, and resilience against biotic and abiotic stresses (Dini-Andreote, 2020; Hardoim et al., 2015). Due to these attributes, many members of these microbiotas are referred to as plant growth promoters (PGPs).

Plant growth promotion occurs through various mechanisms, including enhancing the bioavailability of essential nutrients (e.g., nitrogen, phosphorus, potassium, iron), producing and modulating phytohormones (e.g., ethylene, auxins), and synthesizing biocidal compounds (e.g., antibiotics, surfactants), among other activities (Compant et al., 2010; Etalo et al., 2018). The expression of these mechanisms depends on plant genotype, soil type, plant-microbe interaction specificity, climate conditions, and agriculture practices (Compant et al., 2019; Guttman et al., 2014; Scheres & van der Putten, 2017). These PGP traits stress the significance of studying endophytic microbiotas, particularly for their potential biotechnological applications as bioinputs (biofertilizers, biostimulants, and biopesticides) in agriculture, offering sustainable alternatives to chemical inputs (Battistoni et al., 2023). Moreover, the bacterial microbiotas can modulate the secondary metabolism of the plant, which in turn responds by producing specific compounds of biotechnological importance (Etalo et al., 2018).

Particularly, studies on the microbiota associated with *Cannabis sativa* (cannabis) plants report that its composition and diversity depend on the plant organ (roots, stems, leaves, seeds, flowers, and rhizosphere), as well as the soil type and plant genotype (Winston et al., 2014). Specific bacterial genera (e.g., *Pseudomonas*, *Bacillus*, *Rhizobium*) and fungal genera (e.g., *Alternaria*, *Penicillium*) constitute core microbiomes tailored to specific genotypes and plant compartments (Ahmad et al., 2024; Barnett et al., 2020; Wei et al., 2021). Furthermore, bacterial components of these microbiotas have been reported to improve growth, stress tolerance, and production of bioactive compounds such as cannabinoids and terpenes (Comeau et al., 2021; Lobato et al., 2024; Lyu et al., 2021; Pagnani et al., 2018). These qualities highlight the importance of studying these communities considering the specific characteristics that affect them, such as the genotype of the plant and the conditions under which cannabis plants are grown.

Cannabis is a versatile crop grown in Uruguay primarily for flowers, seeds, and fiber production (Uruguay XXI, 2024). Nevertheless, optimal growth of this crop currently relies on a technological package centered on chemical inputs, which poses significant economic, social, and environmental challenges. From a socio-environmental point of view, there are different adverse effects, including: soil contamination (impacting its health, as well as that of the associated macro- and microbiota); contamination of underground and surface water courses (causing eutrophication); impact on biodiversity (for example, on the health of key pollinators such as bees), and food contamination, compromising its safety. From a socio-economic point of view, agrochemicals or their components are imported, causing loss of profitability and sovereignty (Alcántara et al., 2022; Balbuena et al., 2023; Castelli et al., 2021, 2023; Goyenola et al., 2021; Kruk et al., 2023). Finally, the presence of certain chemical residues, particularly fungicides, can be a significant obstacle, seriously limiting the export of cannabis products to the most stringent and highest-value markets. These impacts stress the urgent need for more sustainable practices in cannabis production. In this context, developing bioinputs based on plant growth-promoting bacteria is particularly relevant for cannabis cultivation. The initial steps in developing such bioinputs involve isolating and characterizing (physiologically and genetically) the bacteria associated with the specific crop, followed by assessing their effects when applied as inoculants.

The aims of this study were to construct and characterize a collection of endophytic bacteria associated with the hemp cultivar Luma, as an initial step to improve the health status, yield, and growth of the plant through its re-inoculation as a bioinput.

2. Materials and Methods

2.1 Plant Samples

Healthy *Cannabis sativa* cv. Luma plants were used as traps for endophytic bacteria isolation. For that, pots containing 1.5 kg of different substrates were used, including 1) soil from a *Cannabis*-producing field; 2) commercial greenhouse soil; and 3) a mixture containing soils 1 and 2 with sterile sand (0.5:0.5:1 ratio). The soil profiles of 1 and 2 were as follows: 1 (pH 5.0, 3.8% organic matter, total N 0.18%), 2 (pH 6.1, 6.6% organic matter, total N 0.33%). Prior to sowing, *C. sativa* cv. Luma seeds were surface-disinfected following described protocols. Briefly, seeds were incubated for 2 minutes in 70% ethanol, washed twice with sterile deionized water, incubated for 10 minutes in 5% NaClO, and finally washed five times using sterile deionized water (Beringer, 1974; Mareque et al., 2015). The surface disinfection process was checked by culturing 100 µL of the last washing water in agar plates containing Tryptic Soy Agar (TSA; Difco Laboratories, Detroit, MI). Surface disinfected seeds were germinated in Petri plates containing water wet sterile cotton (germinator) for 7 days at 26 °C until seedlings emerged. Germinated seeds were sown into pots containing 1.5 kg of substrate and placed in a growth chamber at 26 °C with a photoperiod of 16/8 h light/dark. Plants were watered with tap water as necessary, and harvested for bacterial isolation 20, 40, 90, and 120 days after sowing.

2.2 Isolation of Putative Endophytic Bacteria

Putative endophytic bacteria were isolated from healthy hemp plants seeds, seedlings, roots, and aerial parts. The protocol employed for bacterial isolation was as reported with slight modifications (de los Santos et al., 2016). Before isolation, seeds were surface-disinfected as mentioned above, and plant material was surface-disinfected by two washes in tap water for 20 min, 20 min in 70% ethanol, 15 min in 4% NaClO, and six washes in sterile deionized water.

For bacterial isolation from seeds and seedlings, 2 g of surface-disinfected seeds or seedlings were macerated with mortar and pestle under sterile conditions and resuspended in 9 ml of 0.9% NaCl. For isolation from plant tissues, first, the aerial parts were separated from the plant roots, then pools containing material from five different plants (shoots or roots) were macerated as described above. From all macerated material, 0.1 mL of serial dilutions from 10-2 to 10-5 were plated onto Petri plates containing TSA (DIFCO) and LGI media (Cavalcante & Dobereiner, 1988), and individual colonies showing different morphologies were streaked until pure.

2.3 Screening of the Bacterial Collection for Biofertilization, Phytostimulation, and Colonization Traits

The entire bacterial collection was screened for putative diazotrophs by *nifH*-targeted PCR amplification using the primers PolF and PolR (Poly et al., 2001). The final reaction mixture (25 µl) consisted of 2.5 µl 10× Taq reaction buffer (Fermentas, Thermo Fisher Scientific, Waltham, MA), 3.0 mM $MgCl_2$, 0.16 mM dNTPs, 0.8 µM of both sets of primers, 0.5 U Taq polymerase (Fermentas), 4% bovine serum albumin, and 4.0 µl of a cell lysate template. The PCR amplification program used was the same as described previously (Mareque et al., 2015; Taulé, Zabaleta, et al., 2012). The potential ability to fix N_2 was further tested in vials containing LGI N-free semisolid media, in those isolates that gave a PCR product corresponding to the *nifH* gene (Mirza & Rodrigues, 2012; Reis et al., 1994). The vials were incubated at 30 °C for up to 7 days, and those that showed a growth pellicle were replicated into a new fresh vial containing the same medium (Baldani et al., 2014). Those isolates that both grew as a pellicle in the semisolid N-free media and harbored the *nifH* genes were assumed to be

diazotrophs. In these assays, the strain *Kosakonia radicincitans* UYSO10 was used as a positive control (Taulé, Mareque, et al., 2012).

The abilities to solubilize Fe, P, and K, as well as to produce auxins and enzymes related to the infection process were screened *in vitro* in the entire collection using protocols routinely used in the laboratory (de los Santos et al., 2016; Mareque et al., 2015). Briefly, Fe acquisition by siderophores was evaluated in plates containing chrome azurol medium, incubated for 72 h at 30 °C, with siderophore production visualized as a yellowish area around the colonies (Schwyn & Neilands, 1987). As a positive control the strain *Ensifer meliloti* 1021 was used (Persmark et al., 1993). Phosphate solubilization was evaluated in plates containing GL medium (10 g glucose, 2 g yeast extract, 5 g K2HPO4, 10 g CaCl2, 15 g agar) and incubated for 72 h at 30 °C. Phosphate-solubilizing ability was indicated by the presence of a translucent halo around the colony (positive test result) (Sylvester-Bradley et al., 1982). In this case, the strain *Pantoea dispersa* UYSB45 was used as a positive control (Mareque & Battistoni, 2025). Potassium solubilization was evaluated using Alexandrov medium (10 g sucrose, 1.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1g $CaCO_3$, 15g of agar), and incubated at 30 °C. K-solubilizing ability was indicated by the presence of a translucent halo around the colony (positive test result) (Avakyan et al., 1986). The strain *Enterobacter* sp. UYBN72 was used as a positive control (Ferrari et al., 2023).

For auxin detection, the colorimetric method described by Sarwar and Kremer (1995) was employed. For that the entire collection was grown in a 96-microwell plate containing 2 ml of TSA medium supplemented with 50 mM tryptophan. After 48 h of growth, cultures were centrifuged for 5 min at 12,000 g, and 150 µl of each supernatant were incubated in a 96-microwell plate for 30 min in darkness containing 100 µl of Salkowski reagent. The reaction was followed colorimetrically by measuring the optical density at 540 nm ($O.D._{540nm}$). The same procedure was used to create a calibration curve for IAA ranging from 0 to 1,000 µM. The production of IAA was normalized by measuring the $O.D._{620nm}$ of each culture. Strain *Kosakonia radicincitans* UYSO10 was used as a positive control (Taulé, Mareque, et al., 2012).

Cellulase and hemicellulase activities were screened by growing the isolates on solid TSA culture media supplemented with 0.2% carboxymethyl cellulose or 0.5% Avicel, respectively (Kim et al., 2008). Positive strains were identified by degradation halos around each colony. In these assays the strain *Brevibacillus* sp. UYSB28 was used as a positive control (Mareque et al., 2015). Protease activity was evaluated in plates containing TSA medium supplemented with 5% skimmed milk (Martínez-Rosales & Castro-Sowinski, 2011). Strains were considered to possess protease activity when a translucent halo was observed around the colonies. As a positive control the strain *Rhizobium* sp. UYSB13 was used (Mareque et al., 2015). For the determination of peroxidase activity, the strains were grown in plates containing TSA medium supplemented with 250 mg l^{-1} of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; Sigma-Aldrich, St. Louis, MO); for the determination of manganese (Mn)-peroxidases, the strains were grown on TSA medium with 250 mg l^{-1} ABTS + 100 mg l^{-1} $MnCl_2 \cdot 4H_2O$ (Sack et al., 1997). Strains were considered to possess peroxidase activity when the color of the colonies turned dark green or brown, respectively.

All *in vitro* characterizations were made in triplicate. Differences in the frequency of traits depending on isolation origin were tested using $X2$ tests (Preacher, 2001).

2.4 Physiological Characterization

The entire collection was tested for their ability to use different carbon and nitrogen sources. For that, isolates were grown on plates containing LGI medium supplemented with each nutrient source to test at the concentrations reported in the original protocol (Cavalcante & Dobereiner, 1988). The C sources tested were mannitol, unrefined sugar, maltose, malic acid, pyruvic acid, vinasse, glycerol, lactose, saccharose, fructose, glucose, and ethanol, and the N sources were glutamate, asparagine, urea, potassium nitrate, ammonium sulfate, L-tyrosine, and ammonium chloride.

2.5 Antifungal Activity

Since plant protection from pathogens is likely a selected trait among seed-inhabiting endophytes, all isolates obtained from seeds and seedlings were tested for antifungal activity against *Botrytis cinerea* A1 (Ponce de León et al., 2007). Tests were carried out on petri dishes containing Potato Dextrose Agar (PDA). The fungal inoculum was obtained from a freshly grown culture on PDA and the tested bacteria were inoculated as a 5 µL drop of a cell suspension made on NaCl 0.85% from a fresh culture to a final concentration ranging from 10^5 to 10^6 ufc/mL, with 4 replicates per plate. Plates were incubated at 25 °C and fungal growth was compared to a control plate with no bacterial inoculation. Isolates that generated inhibition zones greater than 1 mm were considered positive for *Botrytis cinerea* inhibition.

2.6 Strain Identification and Phylogenetic Analysis

DNA extraction and PCR amplification of the near full length *16S rRNA* gene were carried out as reported in de los Santos et al. (2016). Sequencing of PCR products was carried out by Macrogen Inc. (Korea). The quality of the sequences obtained was checked and the sequences were assembled using the software DNA Baser Sequence Assembler v3 (version of 2012). Taxonomy was assigned using the NCBI BLAST tool, and the sequences were deposited in GenBank under the following accession numbers: PV786168-PV7886202. In addition, sequences were aligned with Clustal W and the phylogenetic tree was constructed using the Maximum Likelihood method in the Mega X software (Kumar et al., 2018).

3. Results

3.1 Construction and Characterization of a Bacterial Endophytic Collection Associated with Cannabis Plants

A collection of 236 putatively endophytic bacterial isolates associated with *Cannabis sativa* cv. Luma was obtained (Table 1). Most isolates were obtained from the rich TSA medium (n = 205), and only ~ 13% (n = 31) from the N-deficient medium, LGI. From the whole collection, 16 isolates were part of the vertically transmitted microbiome, since they were obtained from seeds (n = 8) and seedlings (n = 8) that had not been in contact with soil. From the plantlet endophytic associated microbiota, 149 isolates were obtained from roots and 71 from leaves and stems (Table 1 and S2). Of the tested substrates, the mixed substrate, containing field soil and sterile sand, provided the best support for plants to grow. Consequently, these plants were grown for longer periods (90 and 120 days) and were the source of most of the bacterial isolates (n = 168) (Table S1).

Table 1. Number of bacterial isolates obtained from different plant zones and on different culture media

Origin [a]	Plants		Medium [b]	
	Roots	Aerial part	LGI	TSA
Seed	-	-	-	8
Seedling	-	-	-	8
Plants (20 d)	21	5	5	21
Plants (40 d)	30	0	11	19
Plants (90 d)	34	19	5	48
Plants (120 d)	64	47	10	101
TOTAL	**149**	**71**	**31**	**205**

[a] Seedlings were germinated 7 days. Plants of 20, 45, 90, and 120 days old were grown on pots with soil in non-sterile conditions.
[b] Medium of isolation: TSA Tryptic Soy Agar, LGI modified Luria-Bertani (LB).

The isolate collection obtained showed great versatility in the use of the different C and N sources evaluated. In this regard, at least 50% of the isolates in the collection were able to grow on the N and C sources evaluated (Table 2), with malic acid and vinasse being the C sources used in the lowest proportion (<50% of the isolates).

Table 2. Availability of putative endophytic bacterial isolates from cannabis (*Cannabis sativa*) cv. Luma to grow in different N and C sources

	Compound	Isolates (%)
N-source	Potassium nitrate	83
	Urea	80
	L-Tyrosine	79
	Asparagine	75
	Glutamate	70
	Ammonium sulphate	60
	Ammonium chloride	50
C-source	Glucose	86
	Sucrose	80
	Maltose	74
	Glycerol	74
	Crystal sugar	73
	Mannitol	70
	Fructose	59
	Pyruvic acid	50
	Lactose	46
	Malic acid	28
	Vinasse	8

All isolates were tested for traits typically associated with direct or indirect PGP and for host colonization. Approximately 10% of the isolates (n=25) showed a positive amplification of the *nifH* gene. Of those, 23 isolates could grow in vials containing LGI N-free semisolid media, making them potential diazotrophs (Figure 1A). In addition, 35% of the isolates (n=84) were able to produce auxin-like compounds in the tested conditions, while 17% of the isolates (n=42) were able to solubilize P, K, and Fe. Of the hydrolytic activities tested, protease activity was the only one widely present, with 17% of the isolates showing positive results (n=42). Only one isolate was positive for cellulolytic activity, while the other activities assayed were not found throughout the members of the collection (Figure 1A).

A total of 44 isolates presented more than one PGP-associated trait, which represents approximately 18% of the isolate collection (Figure 1B). Of those, 26 showed 2 of the tested traits, 5 showed 3 traits, and 3 showed 4 of them, which means that only 2% of the isolates presented 3 of the tested traits.

Some traits were unevenly distributed along plant tissues. Specifically, the proportion of protease producers was significantly higher among isolates obtained from seeds/seedlings (Table S3; X^2 [1, n = 236] = 16.9403, p = 0.00004) than from all other plant parts. In addition, none of the potential N-fixing bacteria were obtained from seeds/seedlings (Table 3), suggesting differential selection of bacterial traits based on the plant growth stages.

In vitro evaluation of the antagonistic activity of all seed and seedling-derived isolates was carried out (Figure S1). From the 16 tested isolates, 12 showed some level of inhibition of the plant pathogen evaluated (Table 3). Interestingly, the four isolates that showed no antagonism were the only ones that did not belong to the

Bacillus genus and were classified as *Massilia*, *Tatumella*, and *Staphylococcus* (2 isolates). Inhibition zones in the growth area of the fungal target ranged between 1 to 6 mm, with an average of 3.75 mm.

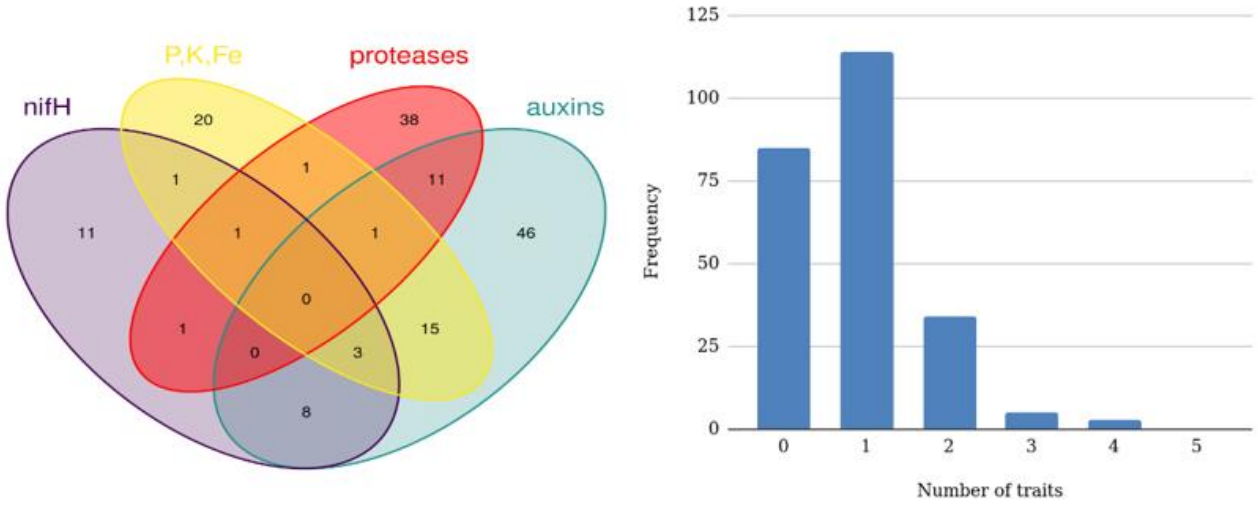

Figure 1. Plant growth promotion-associated traits in the isolate collection

A: Venn diagram showing the number of isolates that have the evaluated traits. B: Frequency distribution of isolates with 0-5 number of the tested traits.

Table 3. Plant growth promotion and infection traits of selected putative endophytic bacterial isolates from cannabis (*Cannabis sativa*) cv. Luma

					Biofertilization and phytostimulation [c]								Infection [d]			Biocontrol [e]
Isolate	**Identification [a]**	**soil**	**Origin**	**Medium [b]**	***nifH***	**GP**	**P**	**K**	**SID**	**IAA**	**CE**	**HC**	**PROT**	**PER**	**Mn-PER**	***Botrytis cinerea* inhibition (mm)**
UYCS1	*Bacillus*	-	seed	TSA	-	-	-	-	-	-	-	-	+	-	-	4
UYCS2	*Massilia*	-	seed	TSA	-	-	-	-	-	-	-	-	+	-	-	0
UYCS3	*Staphylococcus*	-	seed	TSA	-	-	-	-	-	-	-	-	-	-	-	0
UYCS4	*Tatumella*	-	seed	TSA	-	-	+	-	-	+	-	-	-	-	-	0
UYCS5	*Bacillus*	-	seed	TSA	-	-	-	-	-	-	-	-	+	-	-	3
UYCS6	*Staphylococcus*	-	seed	TSA	-	-	-	-	-	-	-	-	-	-	-	ND
UYCS7	*Bacillus*	-	seed	TSA	-	-	-	-	-	-	-	-	+	-	-	5
UYCS8	*Bacillus*	-	seed	TSA	-	-	-	-	-	-	-	-	-	-	-	5
UYCS9	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	+	-	-	-	-	-	1
UYCS10	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	+	-	-	-	-	-	1
UYCS11	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	-	-	-	+	-	-	4
UYCS12	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	+	-	-	+	-	-	3
UYCS13	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	-	-	-	+	-	-	5
UYCS14	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	-	-	-	+	-	-	4
UYCS15	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	-	-	-	+	-	-	4
UYCS16	*Bacillus*	-	seedlings	TSA	-	-	-	-	-	+	-	-	+	-	-	6

					Biofertilization and phytostimulation [c]								Infection [d]			Biocontrol [e]
Isolate	Identification [a]	soil	Origin	Medium [b]	*nifH*	GP	P	K	SID	IAA	CE	HC	PROT	PER	Mn-PER	*Botrytis cinerea* inhibition (mm)
UYCS22	*Ensifer*	1	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS35	*Pseudomonas*	2	roots	LGI	+	+	-	-	-	-	-	-	+	-	-	ND
UYCS44	*Agrobacterium*	1	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS64	*Mycolic bacterium*	1	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS65	*Mycobacterium*	1	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS67	*Pseudomonas*	1	roots	LGI	+	+	+	+	+	-	-	-	-	-	-	ND
UYCS84	*Pseudomonas*	3	roots	TSA	+	+	-	-	-	+	-	-	-	-	-	ND
UYCS110	*Mycolic bacterium*	3	aerial part	LGI	+	+	-	-	-	+	-	-	-	-	-	ND
UYCS115	*Pseudomonas*	3	aerial part	TSA	+	+	-	+	-	+	-	-	-	-	-	ND
UYCS121	*Sinorhizobium*	3	aerial part	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS124	*Shinella*	3	aerial part	TSA	+	+	-	-	-	+	-	-	-	-	-	ND
UYCS127	*Xanthomonas*	3	aerial part	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS146	*Pseudomonas*	3	roots	TSA	+	+	+	-	-	-	-	-	+	-	-	ND
UYCS148	*Pseudomonas*	3	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS149	*Mycolic bacterium*	3	roots	TSA	+	-	-	-	-	+	-	-	-	-	-	ND
UYCS156	*Aminobacter*	3	roots	TSA	+	+	-	-	-	+	-	-	-	-	-	ND
UYCS157	*Xanthomonas*	3	roots	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS158	*Pseudomonas*	3	roots	TSA	+	+	-	+	-	+	-	-	-	-	-	ND
UYCS169	*Paenibacillus*	3	roots	TSA	+	+	-	-	-	+	-	-	-	-	-	ND
UYCS220	*Pseudomonas*	3	aerial part	TSA	+	+	-	-	-	-	-	-	-	-	-	ND
UYCS223	*Microbacterium*	3	aerial part	TSA	+	+	-	+	-	+	-	-	-	-	-	ND
UYCS230	*Staphylococcus*	3	aerial part	TSA	+	+	-	-	-	+	-	-	-	-	-	ND

[a] Taxonomic identification based on *16S rDNA* gene similarity. [b] Medium of isolation: TSA Tryptic Soy Agar, LGI modified Luria-Bertani (LB) medium. [c] *nifH:* *nifH* gene detected by PCR approach, GP: growth pellicle, P: solubilization of inorganic calcium phosphate (Ca_3PO_4), K: potassium solubilization, SID: siderophore production, IAA: indole-3-acetic acid production. [d] CE: exo-cellulase activity; HC: hemicellulase activity; PROT: protease activity, PER: peroxidase, Mn-PER: Manganese-peroxidase. [e] Growth inhibition by dual culture was tested only in seed and seedling isolates. ND: not determined. (+) positive for the trait; (−) negative for the trait.

3.2 Taxonomic Identification of Selected Isolates

A subgroup within the collection was selected for taxonomic identification, including all isolates with *nifH* PCR amplification positive results, and all those obtained from seeds and seedlings. Overall, 40 isolates were identified (Figure 2; Tables 3 and S4), which belonged to the phyla Pseudomonadota, Bacillota, and Actinobacteriota. The most abundant genus of this set of isolates is *Bacillus* (n=12), followed by *Pseudomonas* (n=5). Notably, within this set of identified isolates, 14 of the 16 Bacillota isolates, which belonged to the genera *Bacillus*, *Staphylococcus,* and *Paenibacillus*, were isolated from seeds or seedlings.

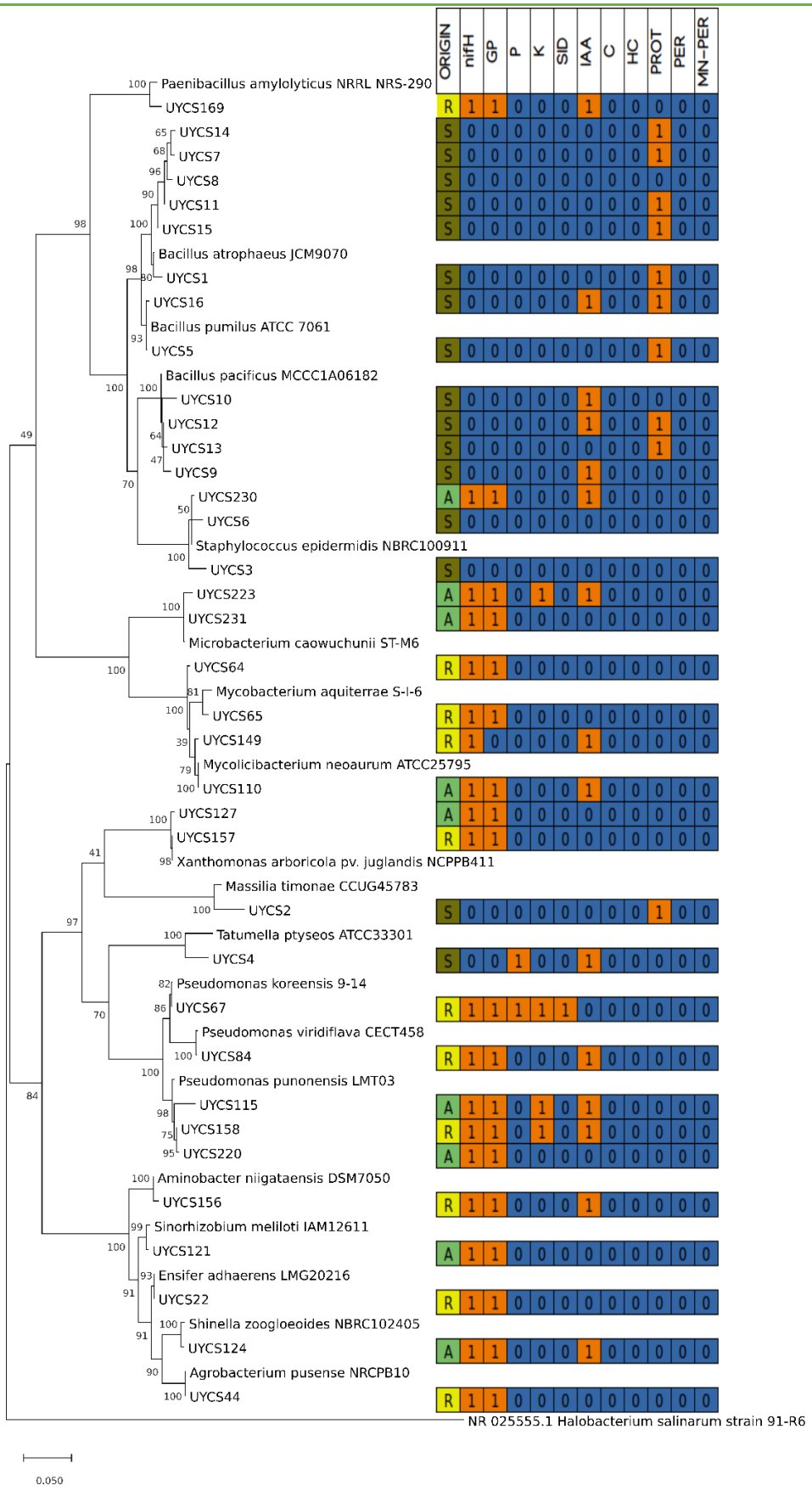

Figure 2. Phylogenetic tree based on sequences of the *rARN 16S* genes of selected isolates inferred from the Maximum Likelihood algorithm using *Halobacterium salinarum* strain 91-R6 as the outgroup

Bacteria of different taxonomic identities are marked within boxes: the phylum Bacillota (previously, Firmicutes) blue, Actinomycetota (previously Actinobacteria) green, Gamma-proteobacteria yellow, and Alpha-proteobacteria red. On the side: R, isolated from roots; S, isolated from seeds or seedlings; A, isolated from aerial parts; 1, presence; 0, absence. Table headings: *nifH*, positive PCR amplification using *nifH* directed primers; GP, growth pellicle in N-deficient medium; P, P solubilization; K, K solubilization; SID, siderophore production; IAA, IAA production; C, cellulase activity; HC, hemicellulase activity; PROT, protease activity.

Among the Pseudomonadota, strains from the classes alpha-beta and gamma-proteobacteria were identified. Among the alpha-proteobacteria, isolates of the genera *Aminobacter*, *Sinorhizobium*, *Ensifer*, *Shinella*, and *Agrobacterium* were identified. Those isolates grouped in well-supported clusters closely related to *Aminobacter niigataensis* DSM7050^T, *Sinorhizobium meliloti* IAM12611^T, *Ensifer adhaerens* LMG20216^T, *Shinella zoogloeoides* NBRC10240^T, and *Agrobacterium pusense* NRCPB10^T strains, respectively. From the gamma-proteobacteria class, isolates of the genera *Xanthomonas*, *Tatumella,* and *Pseudomonas* were identified. Isolates related to the genus *Xanthomonas* were grouped closely related to the *X. arboricola* CFBP 2528^T strain, while isolates related to the genus *Pseudomonas* were grouped in two well-supported clusters, one with isolates closely related to *P. punonensis* LMT03^T strain, and the other with the *P. viridiflava* CECT458^T and *P. koreensis* 9-14^T strains. Regarding the gamma-proteobacteria class, isolates were identified as related to the *Massilia* genus and grouped closely related to the *Massilia timonae* CCUG45783^T strain.

As mentioned, from the phylum Bacillota, identified isolates were related to the *Paenibacillus*, *Bacillus,* and *Staphylococcus* genera (Table 3, Figure 2). Isolates related to the genus *Bacillus* grouped in three well supported clusters closely related to *B. atrophaeus* JCM9070^T, *B. pumilus* ATCC7061^T, and *B. pacificus* MTCC1A06182^T strains, respectively. Additionally, the isolates of the genus *Staphylococcus* were closely related to *S. epidermidis* NBRC100911^T strain, while the isolate of the *Paenibacillus* genus was closely related to *P. amylolyticus* NRRL NRS-290^T strain.

Finally, within the phylum Actinobacteriota, two major clusters were identified: one containing the isolates related to the *Microbacterium caowuchunii* ST-M6^{T}strain, while the other cluster grouped the isolates related to the *Mycolicibacterium neoaurum* ATCC25795^T and *Mycobacterium aquiterrae* S-I-6^T strains.

4. Discussion

The present study reports the construction and characterization of a comprehensive collection of endophytic bacteria associated with *Cannabis sativa* cv. Luma, representing an initial step toward developing sustainable bioinputs for the Uruguayan cannabis industry. The strategy employed herein successfully yielded a collection of 236 isolates obtained from surface-disinfected seeds, seedlings, roots, and aerial tissues of cannabis plants, thereby allowing their classification as putative endophytes. Our results revealed that the isolates within this collection belong to diverse genera, exhibit substantial versatility in utilizing various carbon and nitrogen sources, and display multiple direct and indirect plant growth-promoting (PGP) traits, underscoring their biotechnological potential (Battistoni et al., 2023; Lyu et al., 2023). Several of the genera identified have been described as a part of the cannabis core microbiome (e.g., *Pseudomonas*, *Bacillus*, and *Rhizobium*) and pan-microbiome (e.g., *Xanthomonas*, *Paenibacillus*, and *Staphylococcus*), suggesting that these genera may perform conserved functions relevant to plant-microbe interactions (Barnett et al., 2020). Interestingly, to the best of our knowledge, this is the first report identifying strains belonging to the genera *Mycolicibacterium* and *Aminobacter* as associated with cannabis plants. Members of the genus *Mycolicibacterium*, which belong to the phylum Actinobacteria, are primarily composed of environmental species, and some have been isolated from plants and identified as PGPB (Del Barrio-Duque et al., 2019; Gupta et al., 2018; Saikia et al., 2022). Consistently with the phylogenetic relatedness between the genus *Aminobacter* and the well-known genus *Mesorhizobium*, isolates of this genus have been retrieved from diverse environments –including soils and legume nodules (Mustaq et al., 2024)–, such as *Aminobacter anthyllidis*, a species isolated from nodules of *Anthyllis vulneraria* that elicits functional nodules and enhances plant development under nitrogen-limiting conditions (Maynaud et al., 2012). This observation, together with the finding that the *Aminobacter* sp. isolated herein exhibits potential as a nitrogen-fixing bacterium, justifies further evaluation as a PGP agent.

Notably, the most abundant genera among the identified isolates were Bacillus and Pseudomonas, which is consistent with previous reports on *Cannabis sativa* cultivars (Barnett et al., 2020; Taghinasab & Jabaji, 2020). In the present study, strains related to the genus Pseudomonas were primarily found in roots, whereas those related to Bacillus were predominantly isolated from seeds, emphasizing that microbiome composition is dependent on the plant organ (Scott et al., 2018; Winston et al., 2014). Furthermore, several of these seed-born isolates mentioned exhibited direct and indirect PGP traits, highlighting their importance, as cannabis seed-associated microbiomes demonstrate potential heritability, carrying beneficial bacteria that may influence plant health and productivity (Davies et al., 2024; Lobato et al., 2024).

4.1 Beneficial Potential of the Bacterial Endophytic Microbiome Associated with Cannabis Plants

In vitro analysis of PGP and interaction traits in the bacterial collection revealed that isolates from seeds and seedlings exhibited lower diversity and were predominantly protease producers, potentially reflecting their role in nutrient mobilization during plant germination (Escobar Rodríguez et al., 2020; Verma et al., 2019). These isolates were affiliated with the phylum Bacillota (genera *Paenibacillus*, *Bacillus*, and *Staphylococcus*), which have been previously reported as components of the cannabis seeds microbiome (Davies et al., 2024; Lobato et al., 2024; Scott et al., 2018). Conversely, PGP traits were more prevalent among isolates from roots and aerial tissues, particularly with respect to nitrogen-fixing potential. These isolates were associated with the phyla Actinobacteriota (genera *Mycolicibacterium*, *Mycobacterium*, and *Microbacterium*) and Pseudomonadota (genera *Pseudomonas*, *Massilia*, *Sinorhizobium*, *Ensifer*, *Shinella*, *Agrobacterium*, and *Aminobacter*). Notably, all alphaproteobacteria isolates were both positive for *nifH* gene amplification and capable of growing in N-free semisolid media, strongly supporting their diazotrophic capacity. The bacterial genera obtained in the present study have been previously reported as plant-associated (Ahmad et al., 2024; Barnett et al., 2020; Battistoni et al., 2023; Taghinasab & Jabaji, 2020; Wei et al., 2021). These results underscore the considerable biotechnological potential of this collection with regard to PGP capacity, which may carry significant practical implications. In this sense, cannabis plant growth promotion has been reported following inoculation with strains of the genera *Pseudomonas*, *Bacillus*, and *Paenibacillus*, all of which are well represented in the present collection (Balthazar et al., 2022; Comeau et al., 2021; Lobato et al., 2024; Pagnani et al., 2018). Further experiments are needed to test this capacity, including greenhouse and field inoculation test. It is worth noting that the cannabis-associated endophytes isolated herein may play crucial roles in the biological control of pathogens, as evidenced by the antagonistic activity demonstrated by seed- and seedling-derived bacteria. Furthermore, the ability of certain genera to fix nitrogen, solubilize essential minerals (K, P, Fe), and produce phytohormones could help to reduce reliance on chemical fertilizers in cannabis cultivation through their application as bioinputs. However, it is important to highlight that the PGP effect, following bacterial inoculation, depends on multiple factors, including plant-bacteria genotype compatibility, seed microbiome, soil type, climatic factors, among others (Compant et al., 2019; Guttman et al., 2014; Scheres & van der Putten, 2017). These considerations underscore the importance of conducting context-specific research to identify effective PGP isolates under the particular conditions in which cannabis is cultivated.

In this work, isolates related to the genera *Staphylococcus*, *Agrobacterium*, and *Xanthomonas*, well known as human and plant pathogens, respectively, were also identified. However, those genera have also been described as associated with different cannabis cultivars, highlighting the complexity of the associated microbiota and its potential function (Barnett et al., 2020; Holmes et al., 2023; Scott et al., 2018). In this regard, the presence of a potential pathogenic strain may be related to a specific plant response (Taghinasab & Jabaji, 2020). Such an effect has been reported for example for *Staphylococcus* strains, which modulate the terpenes synthesis in *Catharanthus roses* (Etalo et al., 2018). Moreover, several bacterial endophytes belonging to the classes Gammaproteobacteria, Bacilli and Actinomycetes, which were isolated in this work, have been shown to exert similar modulatory effects in plants, including cannabis, further emphasizing their biotechnology potential (Etalo et al., 2018; Taghinasab & Jabaji, 2020). However, it is important to stress that the isolation of these bacteria from

surface-disinfected, asymptomatic plants may suggest that they may exist as latent endophytes or commensals rather than as active pathogens.

5. Conclusions

Collectively, our results demonstrate that hemp plants harbor a diverse and functionally versatile endophytic microbiome with significant PGP potential and biocontrol capabilities. These findings underscore the importance of microbial research in optimizing cannabis cultivation, with implications for agricultural sustainability, economic efficiency, and the production of high-value compounds. Integrating beneficial microbes into cannabis cultivation practices presents a promising strategy to reduce chemical inputs, enhance soil biodiversity, and support sustainable agricultural systems while maintaining or improving crop yields and bioactive compound production (Balthazar et al., 2022; Comeau et al., 2021; Lobato et al., 2024; Pagnani et al., 2018).

Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author Contribution Statement

	P Vaz-Jauri	C de los Santos	R Platero	F Battistoni
Conceptualization			■	■
Data curation	■	■		
Formal analysis	■	■		
Funding acquisition				■
Investigation	■	■	■	■
Methodology	■	■	■	■
Project administration				■
Resources			■	■
Software	■	■		
Supervision			■	■
Validation			■	■
Visualization	■	■	■	■
Writing – original draft				■
Writing – review and editing	■	■	■	■

References

Ahmad, W., Coffman, L., Ray, R. L., Balan, V., Weerasooriya, A., & Khan, A. L. (2024). Microbiome diversity and variations in industrial hemp genotypes. *Scientific Reports*, *14*(1), Article 29560. https://doi.org/10.1038/s41598-024-79192-7

Alcántara, I., Somma, A., Chalar, G., Fabre, A., Segura, A., Achkar, M., Arocena, R., Aubriot, L., Baladán, C., Barrios, M., Bonilla, S., Burwood, M., Calliari, D. L., Calvo, C., Capurro, L., Carballo, C., Céspedes-Payret, C., Conde, D., Corrales, N., Cremella, B., … García-Rodríguez, F. (2022). A reply to "Relevant factors in the eutrophication of the Uruguay River and the Río Negro". *The Science of the Total Environment*, *818*, Article 151854. https://doi.org/10.1016/j.scitotenv.2021.151854

Avakyan, Z. A., Pivovarova, T. A., & Karavaiko, G. I. (1986). Properties of a new species, *Bacillus mucilaginosus*. *Mikrobiologiya, 55*, 477–482.

Balbuena, S., Castelli, L., Zunino, P., & Antúnez, K. (2023). Effect of chronic exposure to sublethal doses of imidacloprid and nosema ceranae on immunity, gut microbiota, and survival of Africanized honey bees. *Microbial Ecology*, *85*(4), 1485-1497. https://doi.org/10.1007/s00248-022-02014-8

Baldani, J. I., Reis, V. M., Videira, S. S., Boddey, L. H., & Baldani, V. L. D. (2014). The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: A practical guide for microbiologists. *Plant and Soil, 384*, 413-431. https://doi.org/10.1007/s11104-014-2186-6

Balthazar, C., Joly, D. L., & Filion, M. (2022). Exploiting beneficial *Pseudomonas* spp. for cannabis production. *Frontiers in Microbiology*, *12*, Article 833172. https://doi.org/10.3389/fmicb.2021.833172

Barnett, S. E., Cala, A. R., Hansen, J. L., Crawford, J., Viands, D. R., Smart, L. B., Smart, C. D., & Buckley, D. H. (2020). Evaluating the microbiome of hemp. *Phytobiomes Journal, 4*(4), 351-363. https://doi.org/10.1094/PBIOMES-06-20-0046-R

Battistoni, F., Fernández Scavino, A., Ferrando, L., Montañez, A., Pezanni, F., Taulé, C., & Vaz-Jauri, P. (2023). Endophytic and rhizospheric microbial communities associated with native and introduced cultivated plant species in Uruguay as sources for plant growth promotion bioinoculant development. *Environmental Sustainability, 6*, 135–147. https://doi.org/10.1007/s42398-023-00277-6

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). The plant microbiome and its importance for plant and human health. *Frontiers in Microbiology*, *5*, Article 491. https://doi.org/10.3389/fmicb.2014.00491

Beringer, J. E. (1974). R factor transfer in Rhizobium leguminosarum. *Journal of General Microbiology*, *84*(1), 188-198. https://doi.org/10.1099/00221287-84-1-188

Castelli, L., Balbuena, S., Branchiccela, B., Zunino, P., Liberti, J., Engel, P., & Antúnez, K. (2021). Impact of chronic exposure to sublethal doses of glyphosate on honey bee immunity, gut microbiota and infection by pathogens. *Microorganisms*, *9*(4), Article 845. https://doi.org/10.3390/microorganisms9040845

Castelli, L., Branchiccela, B., Zunino, P., & Antúnez, K. (2023). Insights into the effects of sublethal doses of pesticides glufosinate-ammonium and sulfoxaflor on honey bee health. *The Science of the Total Environment*, *868*, Article 161331. https://doi.org/10.1016/j.scitotenv.2022.161331

Cavalcante, V. A., & Dobereiner, J. (1988). A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant and Soil, 108*, 23-31. https://doi.org/10.1007/BF02370096

Comeau, D., Balthazar, C., Novinscak, A., Bouhamdani, N., Joly, D. L., & Filion, M. (2021). Interactions between *Bacillus* Spp., *Pseudomonas* Spp. and *Cannabis sativa* promote plant growth. *Frontiers in Microbiology*, *12*, Article 715758. https://doi.org/10.3389/fmicb.2021.715758

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry, 42*(5), 669-678. https://doi.org/10.1016/j.soilbio.2009.11.024

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research, 19*, 29-37. https://doi.org/10.1016/j.jare.2019.03.004

de los Santos, J., Hawkins, S., Winters, A., & Farrar, K. (2024). Bacterial endophytic community composition varies by hemp cultivar in commercially sourced seed. *Environmental Microbiology Reports*, *16*(2), Article e13259. https://doi.org/10.1111/1758-2229.13259

de los Santos, M. C., Taulé, C., Mareque, C., Beracochea, M., & Battistoni, F. (2016). Identification and characterization of the part of the bacterial community associated with field-grown tall fescue (*Festuca arundinacea*) cv. SFRO Don Tomás in Uruguay. *Annals of Microbiology, 66*, 329-342. https://doi.org/10.1007/s13213-015-1113-2

Del Barrio-Duque, A., Ley, J., Samad, A., Antonielli, L., Sessitsch, A., & Compant, S. (2019). Beneficial endophytic bacteria-*Serendipita indica* interaction for crop enhancement and resistance to phytopathogens. *Frontiers in Microbiology*, *10*, Article 2888. https://doi.org/10.3389/fmicb.2019.02888

Dini-Andreote, F. (2020). Endophytes: The second layer of plant defense. *Trends in Plant Science*, *25*(4), 319-322. https://doi.org/10.1016/j.tplants.2020.01.007

Escobar Rodríguez, C., Antonielli, L., Mitter, B., Trognitz, F., & Sessitsch, A. (2020). Heritability and functional importance of the *Setaria viridis* bacterial seed microbiome. *Phytobiomes Journal, 4*(1), 40-52. https://doi.org/10.1094/PBIOMES-04-19-0023-R

Etalo, D. W., Jeon, J. S., & Raaijmakers, J. M. (2018). Modulation of plant chemistry by beneficial root microbiota. *Natural Product Reports*, *35*(5), 398-409. https://doi.org/10.1039/c7np00057j

Ferrari, E., Taulé, C., Mareque, C., Gonzalez, A., Dourron, J., & Battistoni, F. (2023). Unravelling the plant growth promotion potential of the bacterial endophytic microbiota associated with canola (*Brassica napus*) plants. *Environmental Sustainability, 6*, 403-413. https://doi.org/10.1007/s42398-023-00289-2

Goyenola, G., Kruk, C., Mazzeo, N., Nario, A., Perdomo, C., Piccini, C., & Meerhoff, M. (2021). Producción, nutrientes, eutrofización y cianobacterias en Uruguay: Armando el rompecabezas. *INNOTEC,* (22), Article e558. https://doi.org/10.26461/22.02

Gupta, R. S., Lo, B., & Son, J. (2018). Phylogenomics and comparative genomic studies robustly support division of the genus *Mycobacterium* into an emended genus *Mycobacterium* and four novel genera. *Frontiers in Microbiology*, *9*, Article 67. https://doi.org/10.3389/fmicb.2018.00067

Guttman, D. S., McHardy, A. C., & Schulze-Lefert, P. (2014). Microbial genome-enabled insights into plant-microorganism interactions. *Nature Reviews: Genetics*, *15*(12), 797-813. https://doi.org/10.1038/nrg3748

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293-320. https://doi.org/10.1128/MMBR.00050-14

Holmes, J. E., Sanghera, H., & Punja, Z. K. (2023). Crown gall development on cannabis (*Cannabis sativa* L., marijuana) plants caused by *Agrobacterium tumefaciens* species-complex. *Canadian Journal of Plant Pathology, 45*(5-6), 433-445. https://doi.org/10.1080/07060661.2023.2224776

Johnston-Monje, D., Vergara, L. I., Lopez-Mejia, J., & White, J. F. (2023). Plant microbiomes as contributors to agricultural terroir. *Frontiers in Agronomy, 5*, Article 1216520. https://doi.org/10.3389/fagro.2023.1216520

Kim, S.-J., Lee, C.-M., Han, B.-R., Kim, M.-Y., Yeo, Y.-S., Yoon, S.-H., Koo, B.-S., & Jun, H.-K. (2008). Characterization of a gene encoding cellulase from uncultured soil bacteria. *FEMS Microbiology Letters*, *282*(1), 44-51. https://doi.org/10.1111/j.1574-6968.2008.01097.x

Kruk, C., Segura, A., Piñeiro, G., Baldassini, P., Pérez-Becoña, L., García-Rodríguez, F., Perera, G., & Piccini, C. (2023). Rise of toxic cyanobacterial blooms is promoted by agricultural intensification in the basin of a large subtropical river of South America. *Global Change Biology*, *29*(7), 1774-1790. https://doi.org/10.1111/gcb.16587

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547-1549. https://doi.org/10.1093/molbev/msy096

Lobato, C., de Freitas, J. M., Habich, D., Kögl, I., Berg, G., & Cernava, T. (2024). Wild again: Recovery of a beneficial Cannabis seed endophyte from low domestication genotypes. *Microbiome*, *12*(1), Article 239. https://doi.org/10.1186/s40168-024-01951-5

Lyu, D., Backer, R., Berrué, F., Martinez-Farina, C., Hui, J. P. M., & Smith, D. L. (2023). Plant Growth-Promoting Rhizobacteria (PGPR) with microbial growth broth improve biomass and secondary metabolite accumulation of *Cannabis sativa* L. *Journal of Agricultural and Food Chemistry*, *71*(19), 7268-7277. https://doi.org/10.1021/acs.jafc.2c06961

Lyu, D., Zajonc, J., Pagé, A., Tanney, C. A. S., Shah, A., Monjezi, N., Msimbira, L. A., Antar, M., Nazari, M., Backer, R., & Smith, D. L. (2021). Plant holobiont theory: The phytomicrobiome plays a central role in evolution and success. *Microorganisms*, *9*(4), Article 675. https://doi.org/10.3390/microorganisms9040675

Mareque, C., & Battistoni, F. (2025). Endophytic interaction of the plant growth-promoting strain *Pantoea dispersa* UYSB45 and sweet sorghum (*Sorghum bicolor*) plants. *Plant and Soil, 515*, 407-422. https://doi.org/10.1007/s11104-025-07598-x

Mareque, C., Taulé, C., Beracochea, M., & Battistoni, F. (2015). Isolation, characterization and plant growth promotion effects of putative bacterial endophytes associated with sweet sorghum (*Sorghum bicolor* (L.) Moench). *Annals of Microbiology, 65*, 1057-1067. https://doi.org/10.1007/s13213-014-0951-7

Martínez-Rosales, C., & Castro-Sowinski, S. (2011). Antarctic bacterial isolates that produce cold-active extracellular proteases at low temperature but are active and stable at high temperature. *Polar Research, 30*, Article 7123. https://doi.org/10.3402/polar.v30i0.7123

Maynaud, G., Willems, A., Soussou, S., Vidal, C., Mauré, L., Moulin, L., Cleyet-Marel, J. C., & Brunel, B. (2012). Molecular and phenotypic characterization of strains nodulating Anthyllis vulneraria in mine tailings, and proposal of Aminobacter anthyllidis sp. nov., the first definition of Aminobacter as legume-nodulating bacteria. *Systematic and Applied Microbiology*, *35*(2), 65-72. https://doi.org/10.1016/j.syapm.2011.11.002

Mirza, B. S., & Rodrigues, J. L. (2012). Development of a direct isolation procedure for free-living diazotrophs under controlled hypoxic conditions. *Applied and Environmental Microbiology*, *78*(16), 5542-5549. https://doi.org/10.1128/AEM.00714-12

Mustaq, S., Moin, A., Pandit, B., Tiwary, B. K., & Alam, M. (2024). Phyllobacteriaceae: A family of ecologically and metabolically diverse bacteria with the potential for different applications. *Folia Microbiologica*, *69*(1), 17-32. https://doi.org/10.1007/s12223-023-01107-2

Pagnani, G., Pellegrini, M., Galieni, A., D'Egidio, S., Matteucci, F., Ricci, A., Stagnari, F., Sergi, M., Lo Sterzo, C., Pisante, M., & Del Gallo, M. (2018). Plant growth-promoting rhizobacteria (PGPR) in *Cannabis sativa* 'Finola' cultivation: An alternative fertilization strategy to improve plant growth and quality characteristics. *Industrial Crops and Products, 123*, 75-83. https://doi.org/10.1016/j.indcrop.2018.06.033

Persmark, M., Pittman, P., Buyer, J. S., Schwyn, B., Gill, P. R., Jr., & Neilands, J. B. (1993). Isolation and structure of rhizobactin 1021, a siderophore from the alfalfa symbiont *Rhizobium meliloti* 1021. *Journal of the American Chemical Society, 115*(10), 3950-3956. https://doi.org/10.1021/ja00063a014

Poly, F., Ranjard, L., Nazaret, S., Gourbière, F., & Monrozier, L. J. (2001). Comparison of nifH gene pools in soils and soil microenvironments with contrasting properties. *Applied and Environmental Microbiology*, *67*(5), 2255-2262. https://doi.org/10.1128/AEM.67.5.2255-2262.2001

Ponce de León, I., Oliver, J. P., Castro, A., Gaggero, C., Bentancor, M., & Vidal, S. (2007). Erwinia carotovora elicitors and Botrytis cinerea activate defense responses in Physcomitrella patens. *BMC Plant Biology*, *7*, Article 52. https://doi.org/10.1186/1471-2229-7-52

Preacher, K. J. (2001, April). *Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence* [Software]. https://quantpsy.org/chisq/chisq.htm

Reis, V. M., Olivares, F. L., & Döbereiner, J. (1994). Improved methodology for isolation of Acetobacter diazotrophicus and confirmation of its endophytic habitat. *World Journal of Microbiology & Biotechnology*, *10*(4), 401-405. https://doi.org/10.1007/BF00144460

Rosenberg, E., & Zilber-Rosenberg, I. (2016). Microbes drive evolution of animals and plants: The hologenome concept. *mBio*, *7*(2), Article e01395. https://doi.org/10.1128/mBio.01395-15

Sack, U., Hofrichter, M., & Fritsche, W. (1997). Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of Nematoloma frowardii. *FEMS Microbiology Letters*, *152*(2), 227-234. https://doi.org/10.1111/j.1574-6968.1997.tb10432.x

Saikia, J., Mazumdar, R., & Thakur, D. (2022). Phylogenetic affiliation of endophytic actinobacteria associated with selected orchid species and their role in growth promotion and suppression of phytopathogens. *Frontiers in Plant Science*, *13*, Article 1058867. https://doi.org/10.3389/fpls.2022.1058867

Sarwar, M., & Kremer, R. J. (1995). Determination of bacterially derived auxins using a microplate method. *Letters in Applied Microbiology, 20*(5), 282-285. https://doi.org/10.1111/j.1472-765X.1995.tb00446.x

Scheres, B., & van der Putten, W. H. (2017). The plant perceptron connects environment to development. *Nature*, *543*(7645), 337-345. https://doi.org/10.1038/nature22010

Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, *160*(1), 47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Scott, M., Rani, M., Samsatly, J., Charron, J. B., & Jabaji, S. (2018). Endophytes of industrial hemp (*Cannabis sativa* L.) cultivars: Identification of culturable bacteria and fungi in leaves, petioles, and seeds. *Canadian Journal of Microbiology*, *64*(10), 664-680. https://doi.org/10.1139/cjm-2018-0108

Sylvester-Bradley, R., Asakawa, N., La Torraca, S. M., Magalhães, F. M. M., Oliveira, L. A., & Pereira, R. M. (1982). Levantamento quantitativo de microrganismos solubilizadores de fosfatos na rizosfera de gramíneas e leguminosas forrageiras na Amazônia. *Acta Amazonica, 12*(1), 15-22. https://doi.org/10.1590/1809-43921982121015

Taghinasab, M., & Jabaji, S. (2020). Cannabis microbiome and the role of endophytes in modulating the production of secondary metabolites: An overview. *Microorganisms*, *8*(3), Article 355. https://doi.org/10.3390/microorganisms8030355

Taulé, C., Mareque, C., Barlocco, C., Hackembruch, F., Reis, V. M., Sicardi, M., & Battistoni, F. (2012). The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. *Plant and Soil, 356*, 35-49. https://doi.org/10.1007/s11104-011-1023-4

Taulé, C., Zabaleta, M., Mareque, C., Platero, R., Sanjurjo, L., Sicardi, M., Frioni, L., Battistoni, F., & Fabiano, E. (2012). New betaproteobacterial Rhizobium strains able to efficiently nodulate Parapiptadenia rigida (Benth.) Brenan. *Applied and Environmental Microbiology*, *78*(6), 1692-1700. https://doi.org/10.1128/AEM.06215-11

Uruguay XXI. (2024). *Sector cannabis en Uruguay*. https://www.uruguayxxi.gub.uy/uploads/informacion/0750cc9cb92a1af17373efce4443ee0d0be3538d.pdf

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., & Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *The New Phytologist*, *206*(4), 1196-1206. https://doi.org/10.1111/nph.13312

Verma, S. K., Kharwar, R. N., & White, J. F. (2019). The role of seed-vectored endophytes in seedling development and establishment. *Symbiosis, 78*(2), 107-113. https://doi.org/10.1007/s13199-019-00619-1

Wei, G., Ning, K., Zhang, G., Yu, H., Yang, S., Dai, F., Dong, L., & Chen, S. (2021). Compartment niche shapes the assembly and network of *Cannabis sativa*-associated microbiome. *Frontiers in Microbiology*, *12*, Article 714993. https://doi.org/10.3389/fmicb.2021.714993

Winston, M. E., Hampton-Marcell, J., Zarraonaindia, I., Owens, S. M., Moreau, C. S., Gilbert, J. A., Hartsel, J. A., Kennedy, S. J., & Gibbons, S. M. (2014). Understanding cultivar-specificity and soil determinants of the cannabis microbiome. *PloS ONE*, *9*(6), Article e99641. https://doi.org/10.1371/journal.pone.0099641

Supplementary Material

Table S1. Quantification of the number of colonies obtained in TSA medium according to plant age and tissue and the substrate used for plant growing

Plant age (days)	Quantification (cfu/g) in TSA medium					
	Field soil		Commercial soil		Mix[1]	
	Roots	Aerial part	Roots	Aerial part	Roots	Aerial part
20	10^3	10^2	10^3	10^2	10^3	10^2
40	10^4	10^2	10^4	10^3	10^4	10^3
90	10^4	10^3	10^4	10^3	10^5	10^4
120	10^5	10^4	10^5	10^3	10^6	10^5

[1]Mix: Mixture of field soil, commercial soil, and sterilized sand in a 0.5:0.5:1 ratio.

Table S2. Number of isolates obtained in LGI and TSA media from plants grown in the different substrates

Substrate	Isolation medium		
	TSA	LGI	Total
Field soil	12	4	16
Commercial soil	27	14	41
Mix[1]	149	14	164
Total	**188**	**32**	**220**

[1]Mix: Mixture of field soil, commercial soil, and sterilized sand in a 0.5:0.5:1 ratio.

Table S3. Chi-square contingency table

	Positive	Negative	Total
Seeds + Seedlings	10	6	16
Plant tissues	41	179	220
Total	**51**	**185**	**236**

Table S4. Nucleotide similarity of the *16 ADNr* gene sequences compared to the NCBI database, of hemp plant-associated isolates

Isolate	Best hit	Accession number	Coverage (%)	e-value	Similarity (%)
UYCS1	*Bacillus atrophaeus* strain JCM 9070	NR_024689.1	99	0.0	98.51
UYCS2	*Massilia niabensis* strain 5420S-26	R_044571.1	95	0.0	96.89
UYCS3	*Staphylococcus warneri* strain AW 25	NR_025922.1	94	0.0	98.23
UYCS4	*Tatumella morbirosei* strain LMG 23360	NR_116282.1	96	0.0	94.38
UYCS5	*Bacillus pumilus* strain NBRC 12092	NR_112637.1	100	0.0	96.89
UYCS6	*Staphylococcus epidermidis* strain NBRC 100911	NR_113957.1	99	0.0	98.57
UYCS7	*Bacillus rugosus* strain SPB7	NR_181236.1	99	0.0	98.65
UYCS8	*Bacillus rugosus* strain SPB7	NR_181236.1	99	0.0	98.56
UYCS9	*Bacillus wiedmannii* strain FSL W8-0169	NR_152692.1	99	0.0	96.98
UYCS10	*Bacillus paranthracis* strain MCCC 1A00395	NR_157728.1	100	0.0	98.47
UYCS11	*Bacillus stercoris* strain D7XPN1	NR_181952.1	99	0.0	99.45
UYCS12	*Bacillus wiedmannii strain* FSL W8-0169	NR_152692.1	94	0.0	96.64
UYCS13	*Bacillus wiedmannii* strain FSL W8-0169	NR_152692.1	97	0.0	97.17
UYCS14	*Bacillus rugosus* strain SPB7	NR_181236.1	99	0.0	97.87
UYCS15	*Bacillus cabrialesii* strain TE3	NR_180419.1	99	0.0	98.42
UYCS16	*Bacillus pumilus* strain NBRC 12092	NR_112637.1	99	0.0	98.9
UYCS22	*Ensifer adhaerens* strain NBRC 100388	NR_113893.1	99	0.0	98.81
UYCS35	*Pseudomonas koreensis* strain Ps 9-14	NR_025228.1	99	0.0	99.74
UYCS44	*Agrobacterium fabrum* strain C58	NR_074266.1	99	0.0	99.74
UYCS64	*Mycolicibacterium mucogenicum* strain ATCC 49650	NR_042919.1	99	0.0	99.83
UYCS65	*Mycobacterium aquiterrae strain S-I-6*	NR_158076.1	99	0.0	97.68
UYCS67	*Pseudomonas koreensis* strain Ps 9-14	NR_025228.1	99	0.0	99.42
UYCS84	*Pseudomonas viridiflava*	NR_117825.1	99	0.0	99.16
UYCS110	*Mycolicibacterium neoaurum* ATCC 25795	NR_116556.1	99	0.0	99.23
UYCS115	*Pseudomonas parafulva* NBRC 16636 = DSM 17004	NR_113856.1	99	0.0	95.82
UYCS121	*Sinorhizobium prairiense* strain K101	NR_201879.1	99	0.0	98.97
UYCS124	*Shinella zoogloeoides* strain NBRC 102405	NR_114067.1	99	0.0	99.29
UYCS127	*Xanthomonas maliensis* strain M97	NR_136457.1	99	0.0	99.15
UYCS146	*Pseudomonas viridiflava*	NR_117825.1	99	0.0	98.56
UYCS148	*Pseudomonas parafulva* NBRC 16636 = DSM 17004	NR_113856.1	99	0.0	97.03
UYCS149	*Mycolicibacterium frederiksbergense* strain DSM 44346	NR_025393.1	97	0.0	99.30
UYCS156	*Aminobacter aminovorans* strain DSM 7048	NR_025301.1	68	0.0	95.56
UYCS157	*Xanthomonas campestris* strain ATCC 33913	NR_074936.1	99	0.0	99.83
UYCS158	*Pseudomonas boreofloridensis* strain K13	NR_199512.1	99	0.0	97.85
UYCS169	*Paenibacillus intestini* strain LAH16	NR_156979.1	99	0.0	99.29
UYCS220	*Pseudomonas cremoricolorata* DSM 17059 = NBRC 16634	NR_113855.1	99	0.0	97.85
UYCS223	*Microbacterium oleivorans* strain BAS69	NR_042262.1	99	0.0	98.89
UYCS230	*Staphylococcus epidermidis* strain Fussel	NR_036904.1	99%	0.0	99.28

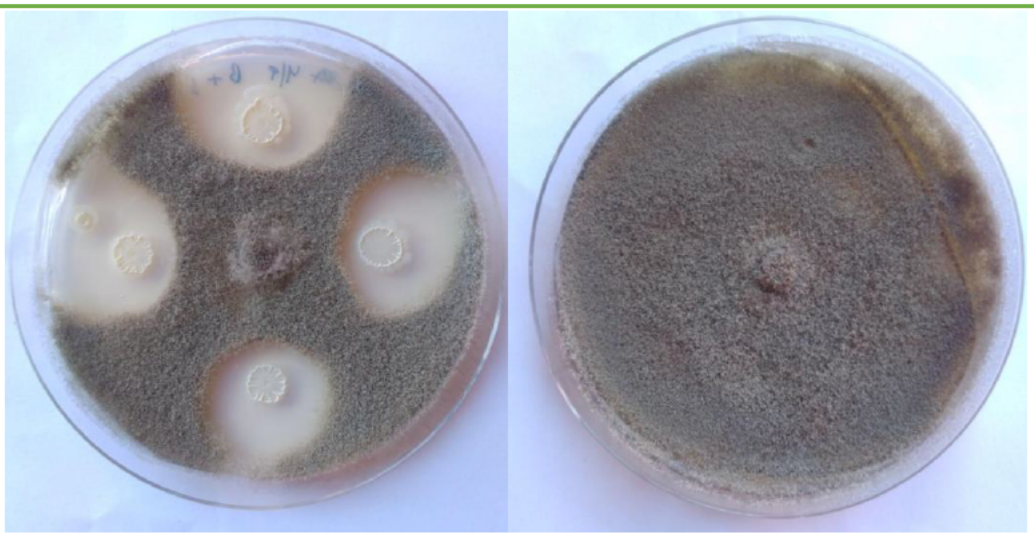

Figure S1. Inhibition assays against *B. cinerea* on PDA medium

Inhibition zones on test plates (left) were measured and averaged. All experiments were compared to a control plate of the fungal target growing without bacteria (right)