Brazilian Journal of Microbiology (2022) 53:1409–1424
https://doi.org/10.1007/s42770-022-00759-5

ENVIRONMENTAL MICROBIOLOGY - RESEARCH PAPER

Diversity and antimicrobial potential of the culturable rhizobacteria from medicinal plant *Baccharis trimera* Less D.C.

Ana Camila Munis Jardim[1] · Jéssica Ellen de Oliveira[1] · Luana de Moura Alves[1] · Giovana Oliveira Gutuzzo[1] · André Luiz Martinez de Oliveira[2] · Elisete Pains Rodrigues[1]

Received: 19 October 2021 / Accepted: 11 April 2022 / Published online: 29 April 2022
© The Author(s) under exclusive licence to Sociedade Brasileira de Microbiologia 2022

Abstract

Plant microbiota is usually enriched with bacteria producers of secondary metabolites and represents a valuable source of novel species and compounds. Here, we analyzed the diversity of culturable root-associated bacteria of the medicinal native plant *Baccharis trimera* (Carqueja) and screened promising isolates for their antimicrobial properties. The rhizobacteria were isolated from the endosphere and rhizosphere of *B. trimera* from Ponta Grossa and Ortigueira localities and identified by sequencing and restriction analysis of the 16S rDNA. The most promising isolates were screened for antifungal activities and the production of siderophores and biosurfactants. *B. trimera* presented a diverse community of rhizobacteria, constituted of 26 families and 41 genera, with a predominance of *Streptomyces* and *Bacillus* genera, followed by *Paenibacillus*, *Staphylococcus*, *Methylobacterium*, *Rhizobium*, *Tardiphaga*, *Paraburkholderia*, *Burkholderia*, and *Pseudomonas*. The more abundant genera were represented by different species, showing a high diversity of the microbiota associated to *B. trimera*. Some of these isolates potentially represent novel species and deserve further examination. The communities were influenced by both the edaphic properties of the sampling locations and the plant niches. Approximately one-third of the rhizobacteria exhibited antifungal activity against *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*, and a high proportion of isolates produced siderophores (25%) and biosurfactants (42%). The most promising isolates were members of the *Streptomyces* genus. The survey of *B. trimera* returned a diverse community of culturable rhizobacteria and identified potential candidates for the development of plant growth-promoting and protection products, reinforcing the need for more comprehensive investigations of the microbiota of Brazilian native plants and habitats.

Keywords Carqueja · Microbiota · Endophytic bacteria · Biosurfactant · Siderophore

Responsible Editor: Lucy Seldin

✉ Elisete Pains Rodrigues
elisete@uel.br

Ana Camila Munis Jardim
acmjbiomed@gmail.com

Jéssica Ellen de Oliveira
jeh.ellen@hotmail.com

Luana de Moura Alves
luanamoura93@gmail.com

Giovana Oliveira Gutuzzo
gi.gutuzzo@gmail.com

André Luiz Martinez de Oliveira
almoliva@uel.br

[1] Laboratório de Genética de Microrganismos, Departamento de Biologia Geral, Universidade Estadual de Londrina, PR-445, Km 380, Campus Universitário, PO Box 6001, Londrina, Paraná CP 86.051-970, Brazil

[2] Laboratório de Bioquímica de Microrganismos, Departamento de Bioquímica e Biotecnologia, Universidade Estadual de Londrina, PR-445, Km 380, Campus Universitário, PO Box 6001, Londrina, Paraná CP 86.051-970, Brazil

Introduction

Healthy plants host a highly complex bacterial community that lives in the rhizosphere and on the surface of and inside the roots and influences the growth, development, physiology, and health of the host plants in different manners [1–3]. The root bacteria community represents a subset of soil bacterial diversity. Bacteria are attracted to the rhizosphere by organic compounds released by the root (rhizosphere effect) sustaining a high density of bacteria and influencing the microbiota composition of this habitat [4]. Some bacteria living in the rhizosphere can colonize the root surface (rhizoplane) and others, known as endophytes, can colonize the root internal tissues (root endosphere) [1]. Since only some rhizobacteria can colonize the root endosphere, the community colonizing this niche represents a subset of that colonizing the rhizosphere, and this, in turn, is a subset of the soil community, the main reservoir from where the bacteria are recruited [4]. The composition of the bacterial community associated with rhizosphere and root endosphere is shaped by a combination of biotic (species, age, developmental stage, and health of plant host) and abiotic factors (soil quality, climate conditions) with the substantial influence of soil type and plant species on the root bacteria community [1, 3, 5].

Plant-associated bacteria affect the plant nutritional status and its tolerance to abiotic and biotic stresses, resulting in improvement in growth, development, health, and productivity of the plant [1, 3, 4, 6]. Living in the rhizosphere and root interface requires certain characteristics from the microbiota, since the rhizosphere is a very competitive habitat where complex microbial interactions, including competition, antagonism, symbiosis, and microbial communication, take place. The competitive interactions and communication with plant hosts and with other members of plant-associated microbiota involve, among other processes, the exchange of distinct secondary metabolites. Thus, the plant-associated microbiota is usually rich in microorganisms with functional capabilities to produce a vast array of secondary metabolites, including antibiotics, toxins, siderophores, and biosurfactants which can influence the establishment of the plant-bacteria interaction [7–9].

The production of siderophores and biosurfactants is a trait observed in many plant-associated bacteria and the antimicrobial properties presented by many of these compounds makes them of great interest in both medical and agricultural fields. Siderophores are iron-chelating agents produced by many bacteria, including plant-associated bacteria, such as *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Azospirillum*, and *Rhizobium*. Siderophores have potential roles and applications in agricultural areas for controlling phytopathogens, enhancing plant growth, and helping in the bioremediation of metal contaminated soil [10, 11]. In the medical field, siderophores can be used to form complexes with antibiotics and help in the selective delivery of antibiotics to the antibiotic-resistant bacteria ("Trojan horse strategy") and in the medical treatment of malaria, cancer, and iron overload diseases [12].

Biosurfactants are microbial derived amphipathic compounds, such as glycolipids, phospholipids, lipopeptides, lipoproteins, and lipopolysaccharides, with tensoactive and emulsifying activities [13]. Production of biosurfactants is a common characteristic in plant-associated bacteria, such as *Pseudomonas*, *Bacillus,* and *Rhizobium* species. Biosurfactants affect important bacterial processes, such as surface motility, biofilm formation, and colonization, which determine the efficiency and success of plant – bacteria interactions [14, 15]. Many biosurfactants present antibacterial, antifungal, antiviral, and antibiofilm properties and are widely used in the pharmaceutical, food, cosmetic, textile, oil, and agricultural industries [16–18]. Considering the potential of plant-associated bacteria to produce biosurfactants and siderophores and/or other compounds with antimicrobial activities, as well as the medical and agricultural relevance of these compounds, the search for plant microbiota must be directed and reinforced to find promising and relevant microorganisms with these abilities.

Endophytic bacteria of medicinal plants serve as an important component of biodiversity and as a promising source of antimicrobial compounds with wide biotechnological applications in the agricultural and pharmaceutical fields [19–21]. The antimicrobial compounds produced by endophytes protect the host plant against attack from pathogens and pests and increase their tolerance to stresses [22]. Among endophytic bacteria, *Streptomyces* is one of the richest sources of antibiotics [23, 24]. *Pseudomonas*, *Bacillus*, *Serratia*, *Azospirillum*, *Burkholderia,* and *Azoarcus* genera also stand out as good producers of antimicrobial substances [25]. *Streptomyces* spp. and their metabolites may have great potential as excellent agents for controlling various fungal and bacterial phytopathogens. Additionally, their ability to promote plant growth has been demonstrated thus expanding the possibilities of using of these bacteria as biofertilizers to increase plant productivity [26]. Despite this, endophytic bacteria of medicinal plants are still relatively poorly investigated and only a few plant species have ever been completely studied [7, 8, 23, 25, 27]. Considering the existence of nearly 300,000 plant species and that each plant can host many endophytes, there is a large opportunity to find new and interesting endophytic microorganisms among myriads of plants in different settings and ecosystems [27].

The Brazilian flora is among the most diverse globally, with a high proportion of endemic plants, many of them with

medicinal properties. Although studies of medicinal plant microbiota are increasing, there are only a few studies with native Brazilian plants and most Brazilian flora still being an unexplored reservoir of new species and biological activities [28]. *Baccharis trimera* (Less) DC (Asteraceae) is a native Brazilian plant, known as carqueja, used to treat diabetes and hepatic and digestive disorders. Carqueja has a wide variety of medicinal properties, including hypoglycemic, hepatoprotective, digestive, antiulcer, anti-acid, antihypertensive, analgesic, anti-inflammatory, antimutagenic, antioxidant, antimicrobial, and antiprotozoal. The insecticide and repellent activities have also been described for *B. trimera* [29].

The natural population of carqueja usually occurs in grassland fields at high altitudes, such as those found in Campos Gerais and Serra do Cadeado in the South region of Brazil. The Campos Gerais comprises the clean fields and natural Cerrado fields located on the second plateau of Paraná State, usually covered by grass and shrubby species with high species richness and endemism [30, 31]. The high diversity and endemism found in these fields, associated with the fact that they are still very little studied, makes these habitats promising for finding new and interesting microorganisms [32]. The microbiota associated with *B. trimera* is still poorly known, and previous studies have focused on endophytic fungi diversity [33]. Since bacteria produce of various secondary metabolites, many of them with antimicrobial properties are abundant in plant habitats, it is interesting to evaluate the antimicrobial potential of the microbiota of *B. trimera*. Here, we characterized the diversity of the bacterial community associated with the roots of *B. trimera* and screened promising isolates for biotechnological applications of agricultural and pharmaceutical interest.

Materials and methods

Soil and plant sampling

The plant samples were collected in two distinct regions of Paraná State, Southern Brazil, in fields covered with native vegetation and no history of agricultural land use. The Ortigueira site is in a place known as "Morro da Pedra Branca" at the Serra do Cadeado (23°57′58″S 51°05′17″W), a field-forest transition zone with a mountainous relief, covered by grass, shrubs, and typical vegetation of mixed ombrophile forest. The Ponta Grossa site is located at the "Dolinas Gêmeas" in the Campos Gerais region (25°08′34″S 49°57′24″W), a mountainous area with rocky outcrops predominantly covered by grass and shrubs. The physicochemical analyses of the soil [34] are given in Online Resource 1. A total of 24 plants were sampled in all the experiment. In each site (Ortigueira and Ponta Grossa), twelve healthy plants were collected and randomly gathered to compose three biological replicates with four samples each (A1, A2, or A3). Samples were transported adequately under refrigeration in plastic bags and processed within 24 h. Brazilian rules to access genetic resources were properly followed.

Counting and isolation of bacteria

Roots (2 g) were vortexed in 50 mL of buffered peptone water (1% peptone, 0.5% NaCl, 0.35% Na_2HPO_4, 0,15% KH_2PO_4; pH 7.4) and sonicated for 30 s in an Elma® ultrasonic water bath (80 kHz, 50% of potency, 4 °C) to obtain the rhizosphere/rhizoplane suspension. The roots were then treated with chloramine-T 1% for 30 min and washed three times with sterilized distilled water. The final washing water was inoculated in potato dextrose agar medium (PDA) in triplicate to evaluate the surface sterilization efficiency [35]. The resulting suspensions were serially diluted in saline solution (1:9 mL v/v). The last three dilutions (0.1 mL) were spread on Reasoner's 2A (R2A) agar medium [36] and potato agar medium without glucose (200 g boiled potato broth and 1.5% agar, pH 6.5), both supplemented with 100 $\mu g.mL^{-1}$ of benzimidazole fungicide. Plates were incubated at 28 °C for up to 30 days, and distinctive colonies were purified on R2A or potato agar medium, after which the cultures were preserved at − 20 °C in tryptic soy broth (TSB) with 50% glycerol. Colonies were counted at 7 days of growth and the colony-forming unit (CFU) was calculated. The statistical analysis was performed with IBM SPSs Statistics software using CFU data transformed to Log10. Data normality was evaluated by the Shapiro–Wilk test and corrected by bootstrapping (1000 bootstraps with 5% confidence interval BCa). The Levene's test ($p < 0.05$) was applied to verify the homogeneity of variances. The analysis of variance (One-way ANOVA) was realized with the Welch's *F*-test and the means were compared with the Games–Howell post hoc test at 5% probability.

DNA isolation and 16S rDNA PCR amplification

DNA was isolated with the phenol–chloroform method, employing mechanical agitation with glass beads [37]. The 16S rDNA was amplified using 27F (5′-AGAGTTTGATCC TGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTT ACGACTT-3′) primers [38] and the GoTaq Green Master Mix (Promega), according to the manufacturer's instructions. Reactions were run in a thermocycler (Amplitherm TX96) at 95 °C for 2 min, followed by 30 cycles of 95 °C for 45 s, 55 °C for 15 s, and 72 °C for 2 min, and a final extension at 72 °C for 10 min. The DNA and PCR fragments were analyzed by agarose electrophoresis (1%), stained with ethidium bromide, and visualized and photographed under ultraviolet light using a Loccus L-PIX photodocumenter.

Amplified 16S rDNA restriction analysis

The 16S rDNA fragments were digested with the endonucleases HaeIII, HinfI, and MspI (Invitrogen), as recommended by the manufacturer. The fragments were separated by electrophoresis on 2% agarose gel at 4 V/cm, stained with ethidium bromide, and photographed. Combined cluster analyses were performed in Bionumerics 6.6 (Applied Mathematics), using the UPGMA (unweighted pair-group method with arithmetic means) algorithm [39] and the Dice similarity coefficient at a tolerance of 2.5%.

16S rDNA sequencing and phylogeny

The 16S rDNA fragments were checked on 1% agarose gels before cleanup with the ethanol-ammonium acetate method [37]. Sequencing was performed on an ABI 3500xL Genetic Analyzer using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) and the 27F primer (20 pmol/μL), according to the manufacturer's instructions. The BioEdit version 7.2.6 was used to check and edit the 16S rDNA partial sequence [40]. The sequencing peak heights of the raw chromatograms were inspected using BioEdit software and then these sequences were manually edited to remove the start and end of the sequences with unreliable base calling and low peak values. Only good-quality sequences with reliable base calling indicated by peak heights in the chromatograms were considered for further analysis.

The multiple sequence alignment and phylogeny were performed on the Phylogeny.fr platform (http://www.phylogeny.fr/index.cgi)[41] and comprised the following steps. Sequences were aligned with MUSCLE (v3.8.31) configured for highest accuracy (MUSCLE with default settings) [42]. After alignment, ambiguous regions (i.e., containing gaps and/or poorly aligned) were removed with Gblocks (v0.91b) using the following parameters: minimum length of a block after gap cleaning equal to 5, minimum number of sequences for a flank position equal to 55%, maximum contiguous nonconserved positions equal to 8, gaps in final block equal to 50% [43]. The phylogenetic tree was reconstructed using the maximum likelihood method and the HKY85 substitution model implemented in the PhyML program (v3.1/3.0 aLRT) [44]. Reliability for internal branch was assessed using the approximate likelihood ratio test support (SH-Like aLRT test [45]. Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) [46, 47]. The 16S rDNA sequences were deposited in the GenBank database under accession numbers from MT233104 to MT233267.

Diversity analysis

The relative abundance (RA%) was determined at family and genera level and used to compare the rhizobacteria communities of the endosphere and rhizosphere of *B. trimera* from Ponta Grossa and Ortigueira. The RA% was calculated by the percentage of the number of isolates of a determined family or genus divided by the total isolates obtained. Abundant families or genera were arbitrarily defined as those with RA > 15% (25 isolates) considering the abundance of the entire data set of families (mean RA of 3.8%) or genera levels (mean RA of 2.4%). Rare families or genera were defined as those with RA 0.6 to 1.8% (1–3 isolates). Families and genera containing mean RA between 2.4 and 6.7% (4–11 isolates) were considered as those with moderate abundance.

The Shannon diversity indices and the hierarchical clustering analyses were performed in PAST 4.05 [48]. The Shannon diversities of each sample (endosphere or rhizosphere) or site (Ponta Grossa or Ortigueira) were compared by the Hutcheson's *t*-test ($p < 0.05$) [49–51] in the PAST software. The clustering analysis was performed using the UPGMA algorithm and Bray–Curtis similarity index with 10,000 bootstraps in PAST 4.05 software [48].

Bioprospection of the isolates

The antifungal activity was tested against the phytopathogens *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides* in PDA medium by dual culture assay [52]. *S. sclerotiorum* causes white mold, a relevant and severe disease of many cultivable plants such as soybean, common bean, and cotton, while *C. gloeosporioides* is the causal agent of anthracnose, a disease of great agronomic importance that causes pre-harvest and post-harvest losses in a variety of vegetables and fruits [53, 54]. The bacteria were inoculated 1 cm away from the petri dish edge, and fungal discs (7 mm) were placed opposite. After 7 days at 28 °C, the fungal inhibition zone was measured and classified as negative (0; no clear zone), low (1; clear zone < 5 mm), moderate (2; 6–14 mm), and high (3; > 15 mm). The phytopathogens were grown in PDA plates in the absence of the bacteria as treatment control. All assays were performed with three replicates.

The siderophores were quantified by the chrome azurol S (CAS) assay [55]. The bacteria were grown for seven days (28 °C, 120 rpm) in 24 deep-well plates with 3 mL/well of Tris-buffered T medium [56] and then centrifuged (3000 rpm, 30 min), and the resulting supernatant was homogenized with CAS reagent (1:1) in 96 well microplates. After 4 h, the absorbance at 630 nm was measured in a PowerWave HT (Biotek) microplate reader. All assays were performed with three replicates. The siderophore concentration was estimated by an EDTA calibration curve

(0–800 µM/mL) and classified as negative (0; no production), low (1; < 300 µM/mL), moderate (2; 300–500 µM/mL), and high (3; > 500 µM/mL).

The production of biosurfactants was analyzed by the drop collapse test [57]. The bacteria isolates were grown for seven days (28 °C, 120 rpm) in 24 deep-well plates with 3 mL/well of 1 × Vogel's salts liquid medium added plus 0.4% glucose. The culture was centrifuged (3000 rpm, 30 min), and supernatant (20 µL) was deposited on the surface of a thin and uniform layer of oil on the lid of a microplate. After 2 min, the drop spreading was analyzed and classified as negative (0; no spreading), low (1; +), moderate (2; ++), and high (3; +++).

Results

Counting and bacteria isolation

Since the CFU data did not follow normal distribution (Shapiro–Wilk = 0.872, $p < 0.006$) and the Levene's test showed an absence of homogeneity of variances (Levene's = 10.279, $p < 0.001$), the means were compared by the Welch's *F*-test and Games–Howell test. For both sites (Ponta Grossa and Ortigueira), the number of cultivable root-associated bacteria of *B. trimera* was significantly lower (Games–Howell test at $p < 0.05$) in the endosphere (6.8 to 7.3 $\log_{10}$ CFU/g fresh weight) than rhizosphere, which had a population about 80 × higher, ranging from 8.2 to 9.3 $\log_{10}$ CFU/g fresh weight. The Ortigueira site had significantly lower bacteria counts (Games–Howell test at $p < 0.05$) than Ponta Grossa in both endosphere and rhizosphere (Fig. 1a).

After CFU counting, the agar plates were visually inspected using a colony counter magnifying lens and colonies with distinct morphologies were selected and transferred onto fresh PDA and R2A media until pure cultures of each isolate were obtained. In the end, we analyzed a total of 274 isolates from the *B. trimera*, mainly from the rhizosphere from the Ponta Grossa site (63.5%) (Fig. 1b). From the visual inspection of agar plates, it was possible to observe that a more diverse set of colonies developed in the R2A than in PDA medium. Approximately 68% of the total isolates were obtained from the R2A medium. These observations together suggest that the R2A was more appropriate for bacteria isolation than PDA, although this observation cannot be confirmed quantitatively.

Restriction and sequencing analyses of the 16S rDNA

The cluster analyses of 16S rDNA restriction patterns revealed the genetic diversity of 274 isolates. The set of isolates was very diverse, totaling 44 distinct groups (72–77% similarity). Most groups were well defined and distinguished the isolates at the genus level. The isolates constituted 206 distinct restriction patterns at 100% similarity level. Additional data are given in Online Resource 2.

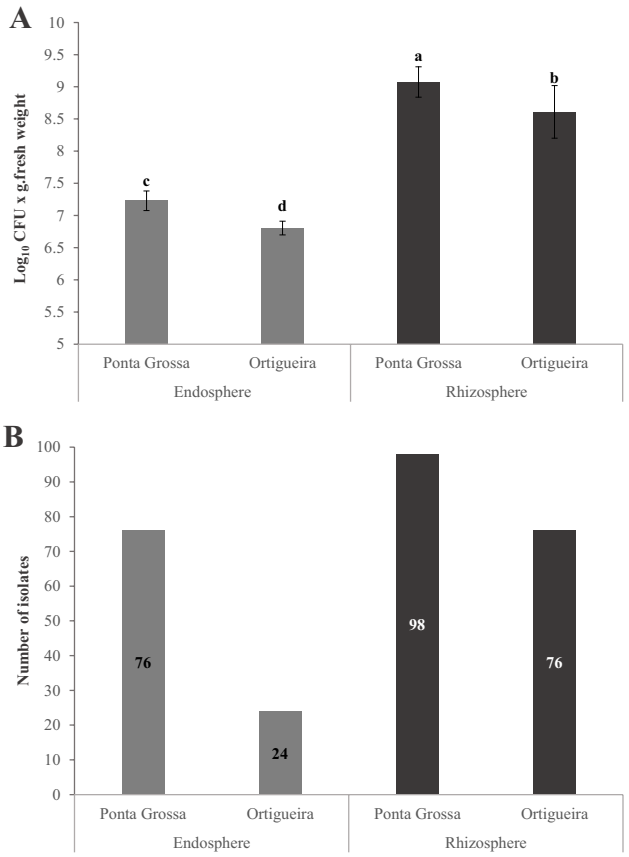

Fig. 1 Bacteria count (**A**) and the number of isolates (**B**) in the root endosphere and rhizosphere soil of *B. trimera* from Ponta Grossa (PG) and Ortigueira (ORT), Paraná State. PDA and R2A, potato agar, and reasoner's 2A agar media. CFU, colony-forming unit. Data are average of three replicates transformed by Log10. Means followed by distinct letters differ statistically (Games–Howell test at $p < 0.05$)

A subset of representative isolates for each amplified 16S rDNA restriction analysis (ARDRA) group was selected for partial 16S rDNA sequencing, totalizing 164 (60%) isolates sequenced. The 164 sequences were deposited under submission numbers MT233104 to MT233267 in the GenBank database as shown in the table (Online Resource 3). The isolates belonged to 26 distinct families of the Actinobacteria, Bacilli, α-Proteobacteria, β-Proteobacteria, and γ-Proteobacteria classes (Fig. 2). The predominant families were Streptomycetaceae (22%) and Bacillaceae (16,5%), followed by Methylobacteriaceae, Paenibacillaceae, Burkholderiaceae, Bradyrhizobiaceae, Rhizobiaceae, and Pseudomonadaceae, with relative abundances (RA) moderate ranging from 4.3 to 6.7%. The families Micrococcaceae, Staphylococcaceae, Xanthomonadaceae,

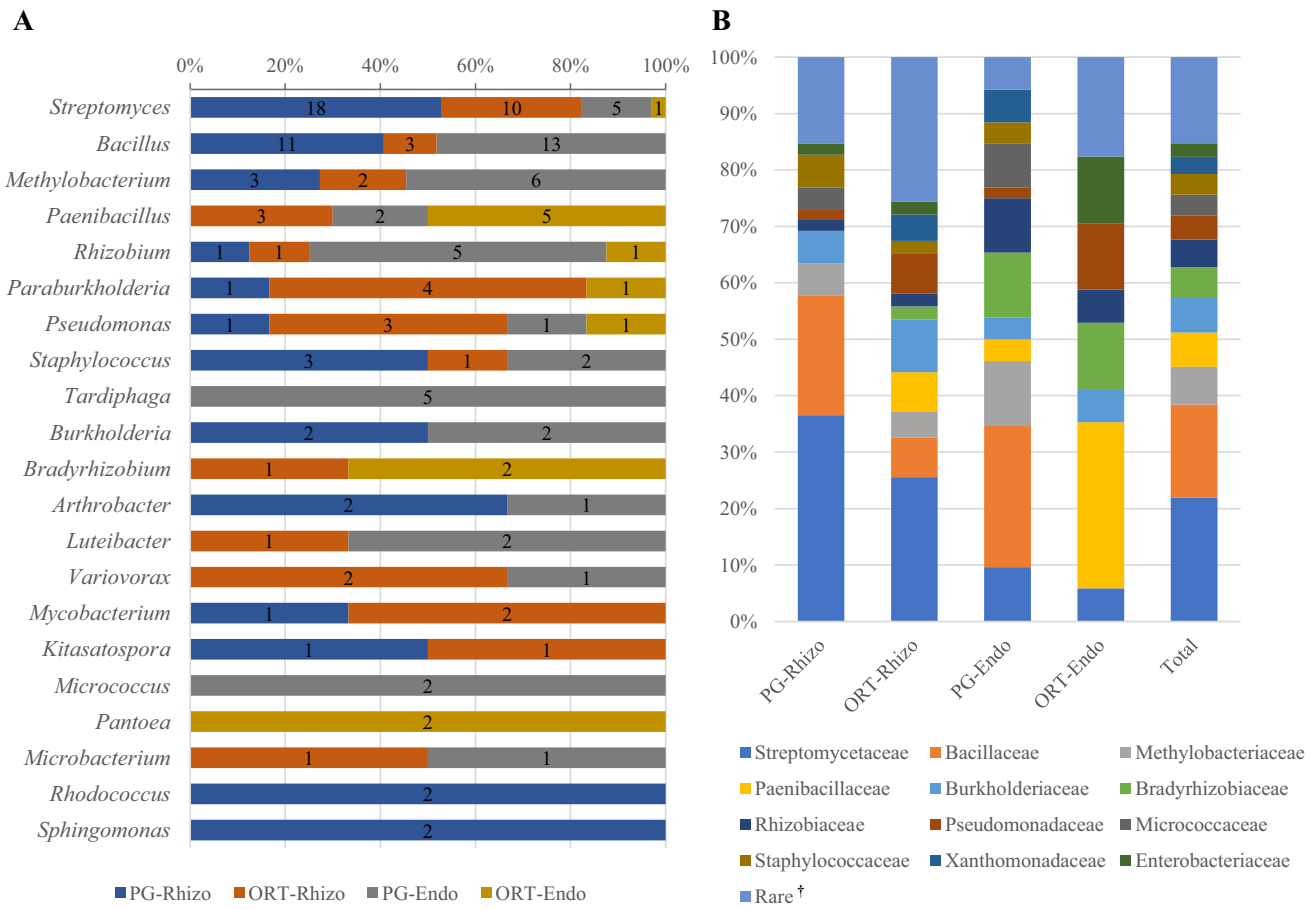

Fig. 2 Relative abundance (RA%) of the main genera (**A**) and families (**B**) of rhizobacteria isolated of endosphere and rhizosphere of *B. trimera* from Ponta Grossa and Ortigueira, Paraná-Brazil. *Rare families include families with RA of 0.6 to 1.8% as detailed in Online Resource 4

and Enterobacteriaceae occurred at moderate RA% of 2.4 to 3.7%. The other fourteen families occurred rarely at relative abundances of 0.6 to 1.8% and corresponded to approximately 15% of the isolates (Fig. 2).

A total of 41 distinct genera were isolated from *B. trimera*. *Streptomyces* and *Bacillus* were the two most abundant genera, followed by *Methylobacterium, Paenibacillus, Rhizobium, Paraburkholderia, Pseudomonas, Staphylococcus*, *Tardiphaga,* and *Burkholderia* that occurred at moderate relative abundance (RA% 2.4 to 6.7). The other 32 genera occurred rarely (RA% of 0.6 to 1.8%) and corresponded to approximately 28% of the isolates (Fig. 2). Details of abundance and distribution of the bacterial classes, families, and genera are given in Online Resource 4.

Comparison of community analysis and diversity index

Figure 3 shows the diversity indices of the bacterial communities. The Shannon indices ranged from 3.67 to 2.83 and were higher ($p < 0.01$) in the rhizosphere than endosphere in both localities (Ponta Grossa and Ortigueira). The diversity index of the rhizosphere was statistically similar ($p < 0.3$) in both localities; however, the diversity index of the endosphere from Ortigueira was significant lower ($p < 0.03$) than Ponta Grossa. The rhizosphere from Ponta Grossa had a high abundance of Actinobacteria and Bacilli isolates, represented mainly by *Streptomyces* and *Bacillus* genera, respectively. Compared to Ortigueira, the bacterial community of the rhizosphere from Ponta Grossa had lesser variety of families and showed predominance of *Streptomyces* and high abundance of the β- and γ-Proteobacteria of the genera *Paraburkholderia* and *Pseudomonas* (Fig. 2).

The diversity of the endosphere from Ponta Grossa was significantly higher ($p < 0.03$) than Ortigueira, and the bacterial composition was also distinct (Fig. 3). The endosphere from Ponta Grossa had a high abundance of *Bacillus* in addition to isolates of *Tardiphaga*, *Methylobacterium*, and *Rhizobium* genera. The endosphere from Ortigueira had a greater abundance of *Paenibacillus*, with five distinct species, besides *Bradyrhizobium* and *Pantoeae* genera, represented by two distinct species (Fig. 2).

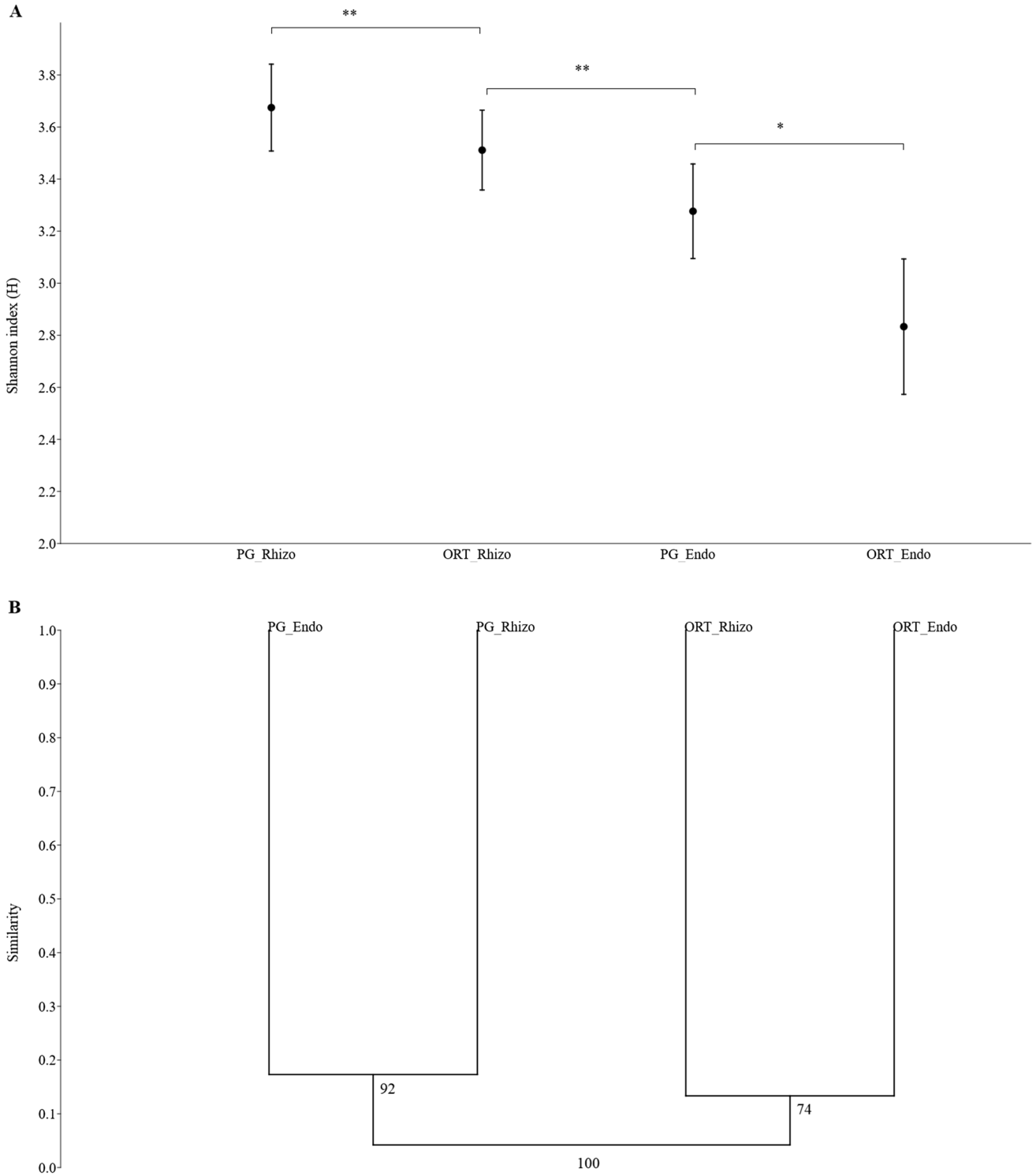

Fig. 3 Shannon diversity indices (**A**) and hierarchical clustering analysis (**B**) for culturable rhizobacteria communities of the endosphere and rhizosphere of *B. trimera* from Ponta Grossa and Ortigueira, Paraná-Brazil. Significant difference at $p \leq 0.05$ (*) or $p \leq 0.01$ (**) by Hutcheson's *t*-test ($p < 0.05$) [49–51]. Clustering used the UPGMA algorithm and Bray–Curtis similarity index with 10,000 bootstraps

The hierarchical cluster analysis showed a separation between the bacteria community from Ponta Grossa and Ortigueira, indicating that the phytogeographic conditions affected the bacterial community structure (Fig. 3). The plant niches were also a relevant factor influencing the composition of the bacterial communities since the Bray–Curtis

similarity between these niches was low (< 0.2) for the two localities. These results confirm the high diversity of genera and species found in these habitats and show that this diversity was influenced by both the plant niches and phytogeographic conditions.

Taxonomic assignment and phylogenetic relationship

We analyzed the phylogenetic relationships of isolates of Actinobacteria, Proteobacteria, and Bacilli phyla and closely related strains (Online Resource 5) to determine the taxonomic classification of the isolates; however, the high similarity of 16S rDNA sequences did not allow reliable identification at species-level for most isolates. Despite this, the closest species of each isolate and their phylogenetic relationships are shown to demonstrate the diversity within each genus. The main genera and species found for each phylum are discussed below and detailed information is given in Online Resource 3 and 5.

Actinobacteria phylum

The isolates of Actinobacteria phylum (54 isolates) comprised 14 distinct genera, including 32 isolates of *Streptomyces*, the predominant genus, and the genera that had 3 isolates each. The other genera found were less common and include *Mycobacterium*, *Arthrobacter, Microbacterium, Micrococcus*, *Kitasatospora*, and *Rhodococcus* (with 2–3 isolates each genus) and the genera *Leifsonia*, *Kocuria*, *Dactylosporangium*, *Nocardioides*, *Nocardia*, and *Conexibacter* with only one isolate each. The similarity coefficients of *Streptomyces* and other isolates of Actinobacteria with their respective closest species were greater than 99%, except for BTM102, BTM382, and BTM06 isolates that had similarity score below 98.4% (Online Resource 3).

The *Streptomyces* isolates were positioned in twenty-one distinct phylogenetic groups; however, most of them contained more than one *Streptomyces* species (Online Resource 5). The isolates BTM291, BTM449, and BTM550 were similar (> 99.6%) with *S. mirabilis* and clustered this species and with *S. olivochromogenes* (> 84% SH-like support), while the isolates BTM446, BTM302, and BTM301 formed, respectively, a concise branch (> 81% SH-like support) with *S. abikoensis, S. atriruber*, and *S. nodosus* species. The high similarity between the 16S rDNA sequences did not allow clear inference at specie level for most isolates. The clusters represented by *S. mirabilis*, *S. libani*, *S. nojiriensis, S. bungoensis*, and *S. graminisoli* were the most common. The two *Kitasatospora* strains (BTM417 and BTM447) formed a cluster with *K. atroaurantiaca* and *K. purpeofusca*. The ML phylogenetic tree of the other isolates of the Actinobacteria phylum is detailed in Online Resource 5.

Bacilli phylum

The 44 Bacilli isolates included members of the genera *Bacillus* (27), *Paenibacillus* (10), *Staphylococcus* (6), and *Lysinibacillus* (1). The sequence similarity of *Bacillus* isolates ranged from 99.2 to 100% (Online Resource 3) and most isolates were identified only at genus level. The *Bacillus* isolates were distributed in ten distinct phylogenetic groups (Online Resource 5). The most common species were *Bacillus pumilus* and *Bacillus safensis*, which formed one large cluster (> 74% SH-like support) with eleven isolates (BTM146, BTM63, BTM104, BTM109, BTM116, BTM148, BTM182, BTM241, BTM340, BTM388). The other well represent group included *Bacillus cereus* and *Bacillus thurigiensis* species, whose strains formed a cluster (100% SH-like support) with BTM346, BTM366, BTM23, BTM26, and BTM17 isolates. The isolates BTM219, BTM331, BTM332, and BTM418 were positioned together with *Bacillus flexus* and *Bacillus megaterium* species (96% SH-like support). The BTM557 clustered with strains of *Bacillus fumariole* species, while the isolates BTM466, BTM50, BTM264, and BTM375 were positioned close to *Bacillus subtilis*, *Bacillus acidiceler*, *Bacillus sporothermodurans*, and *Bacillus niacin*, respectively. The BTM210 was positioned close to *Bacillus senegalensis* and *Bacillus drentensis* species, while BTM183 was positioned close to with *Bacillus muralis* species.

The *Paenibacillus* isolates had similarity coefficients ranging of 96.5 to 99.9% and were positioned in seven distinct phylogenetic clusters (Online Resource 3 and 5). BTM358, BTM119, and BTM479 had, respectively, 96.5%, 97.4%, and 98.7% similarity with *Paenibacillus chartarius*, *Paenibacillus motobuensis*, and *Paenibacillus alginolyticus*. These isolates clustered with their respective closest species. The BTM569 and BTM528 strains clustered with *Paenibacillus terrae* species (100% SH-like support) and the BTM529 strain was positioned together with *Paenibacillus massiliensis* species (100% SH-like support). The BTM534, BTM540, BTM479, and BTM523 strains had high similarity (> 99%) with *Paenibacillus alginolyticus* and were positioned close to this species in a separated branch (99% SH-like support). The BTM454 strain showed similarity to *Paenibacillus agarexedens* (99%) and *Paenibacillus baekrokdamisoli* (98.6%) and clustered with *P. baekrokdamisoli* (99% SH-like support).

The six *Staphylococcus* isolates showed high similarity coefficients (99.3 to 100%) and were positioned in three distinct clusters in the phylogenetic analysis (Online Resource 3 and 5). The BTM82, BTM117, BTM211, and BTM503 strains grouped together to *S. epidermidis* species (74% SH-like support), while the isolates BTM28 and BTM21 clustered (> 93% SH-like support) with *S. pasteuri* and *S. saprophyticus*, respectively.

Proteobacteria phylum

The Proteobacteria phylum included 66 isolates of 24 distinct genera. The phylogenetic tree of α-Proteobacteria isolates included the genera *Methylobacterium* (11), *Rhizobium* (8), and *Tardiphaga* (5) that were the most frequently isolated, as well as isolates of the *Bradyrhizobium*, *Sphingomonas*, *Caulobacter, Dongia, Bosea,* and *Sphingobium* genera that had one to three isolates each genus (Online Resource 5). The *Methylobacterium* isolates showed high similarity coefficients (97.8 to 99.9%) and represented seven distinct phylogenetic branches. BTM541, BTM101, BTM144, and BTM154 clustered with *M. komagatae* (100% SH-like support), the most common *Methylobacterium* species. The BTM157 and BTM145 strains showed, respectively, 97.8% and 99.3% similarity to *Methylobacterium aerolatum* and were positioned close to this species (85% SH-like support). The BTM78 and BTM177 strains clustered (> 73% SH-like support) with *Methylobacterium aquaticum* and *Methylobacterium phyllosphaerae*, respectively. BTM62 and BTM535 had high similarity to *Methylobacterium radiotolerans* (99.7%) and *Methylobacterium platani* (99.4%), respectively, and were positioned close to these species in the phylogenetic tree.

The eight *Rhizobium* isolates showed similarity coefficients greater than 98.9% and were grouped in five distinct phylogenetic branches (Online Resource 3 and 5). The isolates BTM403 and BTM405 formed a clear branch with *Rhizobium grahamii* (93% SH-like support), while BTM505 and BTM476 strains clustered, with *R. larrymoorei* and *R. cellulosilyticum* (> 94% SH-like support), respectively. The isolates BTM47, BTM45, and BTM385 were positioned (100% SH-like support) in a group gathering *Rhizobium lusitanum, Rhizobium tropici*, *Rhizobium rhizogenes*, and *R. miluonense* species. The BTM399 strain had 98.9% similarity with *Rhizobium taeanense*, but formed a separated branch in the phylogenetic tree (92% SH-like support). The five *Tardiphaga* isolates (BTM343, BTM344, BTM402, BTM408, and BTM427) had high similarity (99.3–99.9%) and clustered with distinct *Tardiphaga robiniae* strains in the phylogenetic tree (100% SH-like support).

The phylogenetic analysis of the β-Proteobacteria isolates (Online Resource 5) comprised mainly the genera *Paraburkholderia* (6), *Burkholderia* (4), and *Variovorax* (3). The other β-Proteobacteria genera (*Duganella, Herbaspirillum, Chromobacterium,* and *Thauera*) were less common with one isolate each genus. The similarity coefficients of the isolates of this class ranged of 99.3 to 100%, with exception of the BTM507 and BTM562 strains that had 97.5 and 98.8% similarity with *Niveibacterium umoris* and *Methylibium petroleiphilum* species, respectively (Online Resource 3). The *Paraburkholderia* isolates were positioned in three distinct phylogenetic branches. The isolates BTM456, BTM485, BTM486, and BTM561 clustered with *Paraburkholderia dipogonis* (95% SH-like support), while BTM317 formed a cluster with *Paraburkholderia terrae* strains (94% SH-like support). The *Burkholderia* isolates BTM02, BTM43, BTM131, and BTM27 formed a cluster with distinct strains of *Burkholderia diffusa* and *Burkholderia ambifaria* (> 78% SH-like support). The BTM559 strain had high similarity (> 99.3%) with *Paraburkholderia phenoliruptrix* and *Burkholderia cepacia* strains but was positioned closest to the *Paraburkholderia graminis* in the phylogenetic tree.

The phylogenetic analysis of the λ-Proteobacteria class (Online Resource 5) included members of *Pseudomonas* (6 isolates), *Luteibacter* (3), and *Pantoea* (2), in addition to the genera *Serratia*, *Stenotrophomonas*, *Xanthomonas*, and *Lelliottia*, that had only one isolate each. The similarity coefficients of the isolates of this class ranged of 99.6 to 100% (Online Resource 3). The *Pseudomonas* strain BTM481 formed a clear group with *Pseudomonas alcaligenes* (99% SH-like support), while the other isolates (BTM336, BTM497, BTM484, BTM519, and BTM213) were positioned in a large group that included distinct *Pseudomonas* species as *Pseudomonas putida*, *Pseudomonas rhizosphaerae*¸ *Pseudomonas vancouverensis,* and *Pseudomonas fluorescens*.

Bioprospection for antifungal, biosurfactant, and siderophore producing bacteria

About 30% of the isolates inhibited at some level the growth of the phytopathogen *S. sclerotiorum* and 26.5% of *C. gloeosporioides*. Most antagonist isolates are from the rhizosphere (74%). Twenty-three isolates showed a high level of inhibition against *S. sclerotiorum* and ten against *C. gloeosporioides* (Fig. 4). Most of them were members of *Streptomyces* and, to a lesser extent, of the genera *Burkholderia*, *Paraburkolderia*, *Bacillus*, *Rhodococcus*, or *Pseudomonas*.

Siderophore production ranged from 7 to 752 µM/mL and was detected in 25% of the isolates, most of them isolated from the rhizosphere (63%). Twenty isolates produced over 500 µM/mL (high level) and comprising isolates of *Streptomyces, Bacillus*, *Paraburkholderia*, *Rhizobium*, *Variovorax, Xanthomonas*, and *Staphylococcus* genera (Fig. 4).

The biosurfactant occurred in 47% of the isolates, most of them obtained from the rhizosphere (61%). Among the producers, 26 isolates showed a high level of production detected by complete drop collapse (Fig. 4). These isolates are *Streptomyces*, *Bacillus*, *Pseudomonas,* and *Paenibacillus* genera (2 to 6 isolates), but at least one isolate of *Burkholderia*, *Methylobacterium*, *Mycobacterium*, *Rhizobium*, and *Tardiphaga* also showed high surfactant production.

The scores attributed for the antifungal, siderophore, and biosurfactant capabilities were summed (maximum score of 12), and the isolates ranked to select the most promising isolates. Table 1 shows the ranking of twenty-one isolates, in

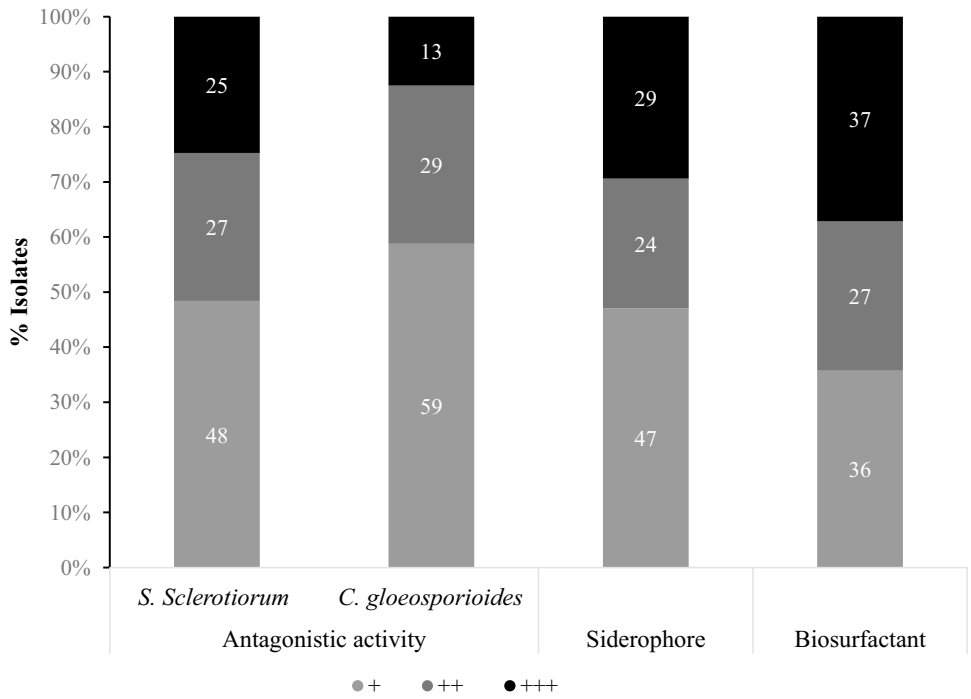

Fig. 4 Bioprospection of rhizobacteria of *B. trimera* for antifungal activity and production of siderophore and biosurfactant. The activity or production was classified as low (+), moderate (++), or high (+++)

which BTM287 showed the highest score (10), followed by BTM295, BTM298, BTM299, and BTM470. *Streptomyces* species and isolates of *Bulkholderia*, *Rhizobium*, *Bacillus*, and *Paraburkholderia* were the top isolates.

Table 1 Ranking of rhizobacteria based on their antifungal activities and production of siderophore and biosurfactant

Isolates	Sclero	Gloesp	Sider	Surf	Score	Rank[†]	Identification[‡]
BTM287	3	3	3	1	10	1st	*Streptomyces* sp.
BTM295	3	1	3	2	9	2nd	*Streptomyces* sp.
BTM298	3	1	2	3	9	2nd	*Streptomyces* sp.
BTM299	3	2	1	3	9	2nd	*Streptomyces* sp.
BTM470	3	3	3	0	9	2nd	*Streptomyces* sp.
BTM27	3	3	1	1	8	3rd	*Burkholderia* sp.
BTM289	2	3	3	0	8	3rd	*Streptomyces* sp.
BTM292	3	3	0	2	8	3rd	*Streptomyces* sp.
BTM120	1	1	2	3	7	4th	*Streptomyces* sp.
BTM294	3	2	2	0	7	4th	*Streptomyces* sp.
BTM02	2	1	0	3	6	5th	*Burkholderia* sp.
BTM20	3	2	1	0	6	5th	*Streptomyces* sp.
BTM43	3	3	0	0	6	5th	*Burkholderia* sp.
BTM47	0	0	3	3	6	5th	*Rhizobium* sp.
BTM210	0	0	3	3	6	5th	*Bacillus soli*
BTM296	2	1	3	0	6	5th	*Streptomyces* sp.
BTM300	2	2	0	2	6	5th	*Streptomyces* sp.
BTM304	3	3	0	0	6	5th	*Streptomyces* sp.
BTM456	3	2	0	1	6	5th	*Paraburkholderia* sp.
BTM463	1	2	3	0	6	5th	*Streptomyces* sp.
BTM471	3	3	0	0	6	5th	*Streptomyces* sp.

[†]Ranking based on activity level as low (1), moderate (2), or high (3)

[‡]Identification based on sequencing or restriction analysis of the 16S rDNA

Sclero, *Sclerotinia sclerotiorum*; Gloesp, *Colletotrichum gloeosporioides*, Sider, siderophore Surf, Biosurfactant

Discussion

Research with plant-associated bacteria has indicated that plants shelter a diverse bacterial community with different biological activities and the potential to produce different secondary metabolites [7, 8, 23, 25]. We analyzed the diversity and antimicrobial properties of rhizobacteria of the carqueja grown in Paraná State, Brazil, and identified a highly diverse community constituted of 274 isolates, belonging to three phyla, five classes, 26 families, 41 genera, and at least 113 species. To our knowledge, this is the first study providing information about the diversity of bacteria associated with *B. trimera*, although the endophyte fungal community has been the subject of a previous study [33]. The biodiversity of microbiota associated with Brazilian medicinal plants is still little known. A recent review study reported that approximately 54 plant species (30 distinct families) were analyzed for endophyte biodiversity in Brazil, with the most representative families demonstrating obvious agronomic and industrial importance, and only a few studies included medicinal plants [28].

The genera most frequently isolated from *B. trimera* rhizosphere and endosphere were *Streptomyces* and *Bacillus*, representing about 37% of all isolates, followed by *Paenibacillus, Staphylococcus*, *Methylobacterium*, *Rhizobium*, *Tardiphaga*, *Paraburkholderia, Burkholderia*, and *Pseudomonas* that occurred at moderate relative abundance (RA of 2.4 to 6.7%). Like other Actinobacteria, *Streptomyces* is a common genus of soil bacterium and can reside in the rhizosphere and inside plant tissues. This genus is known as the main producer of antimicrobial compounds [24]. Studies have reported that these bacteria have various properties in common with other plant-growth-promoting bacteria (PGPB) and can benefit plant growth and productivity when inoculated alone or in a consortium [24, 26, 58]. Our results showed that the *Streptomyces* isolates were the main antagonists of *S. sclerotiorum* and *C. gloeosporioides* and that some isolates were also good producers of biosurfactants (BTM299, BTM298, and BTM293) and siderophores (BTM287, BTM295, and BTM470) (Table 1). Medicinal plants are a reservoir of new and interesting *Streptomyces* species [23]. We found 32 isolates of *Streptomyces* sp. associated with *B. trimera*, which were positioned in 26 distinct branches in the phylogenetic tree. Since the 16S rRNA phylogenetic tree provided a useful framework for the relationships among species but did not always have sufficient resolution to provide definitive identification at species-level [58], the multilocus sequence analysis (MLSA) of the *Streptomyces* isolates is in progress in our laboratory to determine the species of these isolates and if they potentially represent new species.

The Bacilli class was also well represented in the microbiota of *B. trimera* with three genera (*Bacillus*, *Paenibacillus*, and *Staphylococcus*) among the most frequently isolated. Firmicutes, Proteobacteria, and Actinobacteria are among the most frequent phyla isolated from plants and, as we also observed, *Bacillus* is usually the most predominant genus [59]. *Bacillus* was the most frequent genus identified in 77% of articles studying endophytic bacteria of plants grown in Brazil [28].

Bacillus and *Paenibacillus* species can provide a broad range of benefits for plants, including preventing and controlling diseases caused by pathogens, eliciting plant resistance, and promoting plant growth [60, 61]. Due to their beneficial effects on plants and their ability to produce endospores resistant to adverse environmental conditions, interest in *Bacillus*-based inoculant formulations has significantly increased worldwide [62, 63]. Our *Bacillus* isolates showed potential to inhibit the phytopathogen *S. sclerotiorum* (BTM146, BTM148, and BTM241) and to produce siderophores (BTM330, BTM340, and BTM210) and biosurfactants (BTM109, BTM198, and BTM210) at high levels, while the *Paenibacillus* isolates stood out for the high production of biosurfactants (BTM119, BTM523, BTM528, and BTM540) and the capacity to inhibit at a moderate level the growth of phytopathogens (BTM528, BTM569, and BTM570).

The microbiota of *B. trimera* also has a high prevalence of known plant-beneficial bacteria of the Proteobacteria phylum, such as *Methylobacterium*, *Rhizobium*, *Tardiphaga*, *Bradyrhizobium*, *Burkholderia*, *Paraburkholderia*, and *Pseudomonas*. *Methylobacterium* is found on the plant surfaces and as an endophyte of different plant species and can promote plant growth and protection using several mechanisms [64]. In addition to playing a role in plant growth, *Methylobacterium* bacteria can degrade toxic compounds and tolerate high heavy metal concentrations and so can be used to decontaminate the environment [64]. The *Methylobacterium* isolates BTM541 and BTM157 showed moderate antagonistic activity and BTM535 had high production of the biosurfactant. We found at least seven distinct *Methylobacterium* species in the rhizosphere and endosphere of *B. trimera*, including four isolates that were closely related to those of *M. komagatae*, a promising rhizobacteria inoculant for oleaginous Crambe plants [65].

The *Rhizobium* and *Bradyrhizobium* genera are endosymbionts usually isolated from root nodules of leguminous plants, while *Tardiphaga* is a new genus in the Bradyrhizobiaceae family described as a nodule symbiont of *Robinia pseudoacacia* and *Vavilovia formosa* [66, 67]. Here, we identified five *Rhizobium*, three *Bradyhizobium*, and five *Tardiphaga* strains closely related to *T. robiniae* LMG 26467 T. These rhizobia were mainly isolated from the root endosphere of *B. trimera*. *Rhizobium* and *Bradyhizobium* species are endophytes of non-legume hosts such as sugarcane, maize, and wheat [68–70], and novel species of

endophyte rhizobia have been described [71, 72]. Due to their ability to provide nitrogen fixed to legume hosts, symbiotic species such as *B. japonicum* and *R. tropici* have high economic relevance for soybean and common bean crops. There is yet little knowledge about the *Tardiphaga* genus. The *Rhizobium* strain BTM47 showed high capacity to produce siderophores and biosurfactants, while the *Tardiphaga* isolates BTM343, BTM344, BTM402, and BTM427 stood out to produce biosurfactants. As crops, medicinal plants can benefit from interactions with PGPB [73], and further investigations of the role of these rhizobia endophytes in the growth and health of *B. trimera* are needed.

The other more prevalent genera associated with *B. trimera* were *Burkholderia*, *Paraburkholderia,* and *Pseudomonas*. The members of *Paraburkholderia* and *Burkholderia* genera occur commonly in soil, water, and in association with plant, fungi, animal, and humans [74, 75]. The *Burkholderia* genus includes both plant-beneficial and phytopathogenic species, and some species of *Burkholderia* have demonstrated some opportunistic infection to animal and human. Most of plant-beneficial *Burkholderia* species have demonstrated promising biocontrol action against different phytopathogens and significant biotechnological potential as a source of novel antibiotics and bioactive secondary metabolites [75]. The *Paraburkholderia* genus include many beneficial species with potential use in the plant protection and environmental bioremediation [74, 75]. The *Paraburkholderia* genus is prevalent in edaphic adverse conditions, such as those found in the "Campos Gerais" region from Paraná State, characterized by nutrient-poor and acidic soils with high levels of aluminum [31]. The *Paraburkholderia* strains were obtained predominantly from *B. trimera* grown in the Ortigueira and are closely related to *P. phytofirmans*, a beneficial bacterium that promotes the growth and health of various plant species even under stressful conditions [76]. The *Paraburkholderia* isolate BTM317 produced siderophores at a high level, while *Paraburkholderia* isolate BTM456 and *Burkholderia* isolates BTM02, BTM27, BTM43, and BTM131 stood out for the antagonistic activity, inhibiting the growth of phytopathogens at a high level.

We also identified six distinct *Pseudomonas*, including isolates that were closely related to those of fluorescent species *P. migulae*, *P. corrugate*, and *P. aeruginosa*. Many fluorescent *Pseudomonas* species are plant growth-promoting rhizobacteria (PGPR), improving plant health and fitness through a wide range of plant-beneficial traits, including the production of antifungal metabolites. These properties, associated with their high catabolic adaptability and root-colonizing abilities, make these bacteria suitable for biotechnological application in agriculture, especially as an attractive biocontrol agent [77]. In our analyses, the *Pseudomonas* isolates BTM519 and BTM336 were good producers of biosurfactants and siderophores, while BTM481 had antifungal activity against *S. sclerotiorum*.

Besides the ten most predominant genera discussed above, *B. trimera* habitats sheltered another 31 bacterial genera (almost one-third of total isolates) that occurred at low relative abundances. These data show how suitable these habitats are and that more efforts are needed to cultivate these bacteria to have a more representative view of the bacterial community associated with *B. trimera*. Here, poor culture media (mainly the R2A agar) associated with the long incubation time and the sampling in localities with different soil and phytogeographic properties provided a favorable setting for successful isolation of a diverse bacterial collection. In another study, the successful recovery of uncultured and novel bacteria isolates from forest soil was achieved using a diffusion bioreactor associated with a prolonged incubation period, low substrate-modified media, and sampling in summer [78]. Considering that conventional techniques recover only a tiny part of the bacteria community, new and distinct cultivation strategies are necessary to reveal a still hidden bacterial community and discover their potential biotechnological applications [79].

The diversity analyses showed that the bacterial community associated with *B. trimera* is highly diverse, especially in the rhizosphere samples with higher Shannon diversity indices than the endosphere niches for both localities. In addition, the analyses suggest that the composition of the bacteria community associated with *B. trimera* was influenced by both plant niches and phytogeographic conditions. Our results agree with other previous studies that show that a combination of biotic (species, age, developmental stage, and health of plant host) and abiotic factors (soil quality, climate conditions) shape the composition of the bacterial community associated with rhizosphere and root endosphere [1, 3, 5]. In the plant-bacteria interaction process, root-associated bacteria are attracted to the rhizosphere by the root exuded organic compounds (rhizosphere effect), which sustain a highly dense and diverse bacterial community. The host plant's nutritional and physiological status affects the composition and quantity of root exudates, influencing the microbial community [4]. Since the edaphic is a relevant factor influencing plant physiology and nutrition, it is reasonable that the distinct edaphic and phytogeographic conditions found in Ponta Grossa and Ortigueira are a prevalent factor shaping the bacterial community associated with *B. trimera*. The lower diversity and density of bacteria found in the endosphere of *B. trimera* also supports previous findings in the literature [2, 4].

Soil and plant habitats are primary sources of bacteria for various biotechnological applications, particularly for developing products to improve plant growth and productivity and biocontrol of plant disease. Unexplored sources, such as the various medicinal and native plants species, have proven to be a precious reserve of new microbial species for the search for new products and metabolites of agricultural, pharmaceutical, and industrial interest. Here, we identified that approximately

one-third of the rhizobacteria of *B. trimera* exhibited antifungal activity against *S. sclerotiorum* and *C. gloeosporioides*, some of them with high inhibition levels, such as the BTM287 strain. Furthermore, we identified a high proportion of isolates able to produce siderophores and biosurfactants, compounds with antimicrobial activities. The ranking based on antimicrobial properties allowed the selection of the top isolates. Additional studies are needed to determine whether the compounds produced are new molecules. The most promising isolates are members of the *Streptomyces* genus, revealing that the microbiota of *B. trimera* is a source of new and valuable isolates of this genus, as previously reported for other medicinal plant species. Further studies including plant inoculation assays with the most promising isolates are needed to confirm the potential of these isolates as plant growth-promoting and fungal biocontrol agents.

The survey of the medicinal plant *B. trimera* returned a diverse community of culturable rhizobacteria, some of them being potentially new species, considering the sequence identity threshold ≥ 97% established for a definition of a bacteria species [80] and the positioning of the isolates in the ML phylogenetic tree. Between these are the included Actinobacteria strains BTM102 and BTM382 that are, respectively, closely related to *Conexibacter arvalis* and *Mycobacterium komossense* species. Potential new species also included bacteria isolates of the *Paenibacillus* (BTM358 and BTM119) genus and of distinct genera of the Proteobacteria class as *Methylobacterium* (BTM175), *Dongia* (BTM496), and *Niveibacterium* (BTM507). The sequence identity of these isolates with their closest relative species was ≤ 97% (Online Resource 3). The species defining of these isolates needed to be further confirmed using methods with higher resolution at species level as Multilocus sequence analysis (MLSA) [81]. The promising biotechnological potential of some of our isolates added to the fact that some of these isolates represent potentially new species highlights and reinforces the need for more comprehensive investigations of the microbiota of Brazilian native plants and habitats.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-022-00759-5.

Acknowledgements The authors are grateful to Ph.D. Adriana Pereira da Silva for her assistance in the physicochemical analysis of the soil.

Author contribution All authors contributed to the study conception and design or the acquisition, analysis, or interpretation of data. Material preparation, data collection, and analysis were performed by Ana Camila Munis Jardim, Jéssica Ellen de Oliveira, Luana de Moura Alves, Giovana Oliveira Gutuzzo, André Luiz Martinez de Oliveira, and Elisete Pains Rodrigues. The first draft of the manuscript was written by Ana Camila Munis Jardim and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Partial financial support was received from the National Institute of Science and Technology, INCT-Plant-Growth Promoting Microorganisms for Agricultural Sustainability and Environmental Responsibility (CNPq 465133/2014–4, Fundação Araucária-STI 043/2019, CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPq 480080/2013–5). This work was also supported by the infrastructure of the LAMM Facility from Londrina State University. Ana Camila Munis Jardim and Luana de Moura Alves received MSc scholarships from the National Council for the Improvement of Higher Education (CAPES)—Finance code 001. Jéssica Ellen de Oliveira and Giovana Oliveira Gutuzzo received a BASc scholarships from the Araucaria Foundation.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The study does not involve any human or animal participation or data.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Compant S, Vacher C (2019) Sources, niches and routes of colonization by beneficial bacterial endophytes. In: Schouten A, ed. Endophyte Biotechnology: Potential for Agriculture and Pharmacology CABI. 32–41. https://doi.org/10.1079/9781786399427.0032
2. Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486. https://doi.org/10.1016/j.tplants.2012.04.001
3. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64(1):807–838. https://doi.org/10.1146/annurev-arplant-050312-120106
4. Bakker PAHM, Berendsen RL, Doornbos RF, Wintermans PCA, Pieterse CMJ (2013) The rhizosphere revisited: Root microbiomics. Front Plant Sci 4(5). https://doi.org/10.3389/fpls.2013.00165
5. Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68(1):1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x
6. Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J Adv Res 19:29–37. https://doi.org/10.1016/j.jare.2019.03.004
7. Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37. https://doi.org/10.1016/j.copbio.2013.09.012
8. Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7(SEP):1538. https://doi.org/10.3389/fmicb.2016.01538

9. Lareen A, Burton F, Schäfer P (2016) Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90(6):575–587. https://doi.org/10.1007/s11103-015-0417-8
10. Garg G, Kumar S, Bhati S (2021) Siderophore in plant nutritional management: role of endophytic bacteria. 315–329. https://doi.org/10.1007/978-3-030-65447-4_14
11. Fadiji AE, Babalola OO (2020) Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. Front Bioeng Biotech 8:467. https://doi.org/10.3389/FBIOE.2020.00467/BIBTEX
12. Saha M, Sarkar S, Sarkar B, Sharma BK, Bhattacharjee S, Tribedi P (2015) Microbial siderophores and their potential applications: A review. Environ Sci Pollut Res 23(5):3984–3999. https://doi.org/10.1007/S11356-015-4294-0
13. Rani M, Weadge JT, Jabaji S (2020) Isolation and characterization of biosurfactant-producing bacteria from oil well batteries with antimicrobial activities against food-borne and plant pathogens. Front Microbiol 11:64. https://doi.org/10.3389/FMICB.2020.00064/BIBTEX
14. Primo ED, Ruiz F, Masciarelli O, Giordano W (2015) Biofilm formation and biosurfactant activity in plant-associated bacteria. 337–349. https://doi.org/10.1007/978-3-319-24654-3_13
15. D'aes J, de Maeyer K, Pauwelyn E, Höfte M (2010) Biosurfactants in plant–*Pseudomonas* interactions and their importance to biocontrol. Environ Microbiol Rep 2(3):359–372. https://doi.org/10.1111/J.1758-2229.2009.00104.X
16. Singh P, Rale V (2022) Applications of microbial biosurfactants in biocontrol management. Biocontrol Mechanisms of Endophytic Microorganisms. 217–237. https://doi.org/10.1016/B978-0-323-88478-5.00009-2
17. Goswami M, Deka S (2021) Biosurfactant-mediated biocontrol of pathogenic microbes of crop plants. Biosurfactants for a Sustainable Future. 491–509. https://doi.org/10.1002/9781119671022.CH22
18. Naughton PJ, Marchant R, Naughton V, Banat IM (2019) Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. J Appl Microbiol 127(1):12–28. https://doi.org/10.1111/JAM.14243
19. Chandrakar S, Gupta AK (2015) Antibiotic potential of endophytic actinomycetes of medicinal herbs against human pathogenic bacteria. Proceed Nat Academy Sci India Sect B Bio Sci 87(3):905–915. https://doi.org/10.1007/S40011-015-0668-9
20. Elsebai MF, Tejesvi MV, Pirttilä AM (2014) Endophytes as a novel source of bioactive new structures. Adv Endophytic Res 191–202. https://doi.org/10.1007/978-81-322-1575-2_10
21. Barman D, Bhattacharjee K (2019) Endophytic bacteria associated with medicinal plants: the treasure trove of antimicrobial compounds. 153–187. https://doi.org/10.1007/978-981-13-9566-6_8
22. Mohamad OAA, Ma JB, Liu YH, Li L, Hatab S, Li WJ (2019) Medicinal plant-associated microbes as a source of protection and production of crops. 239–263. https://doi.org/10.1007/978-981-13-9566-6_10
23. Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 108(2):267–289. https://doi.org/10.1007/s10482-015-0502-7
24. Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) *Streptomyces* as a plant's best friend? FEMS Microbiol Ecol 92(8). https://doi.org/10.1093/femsec/fiw119
25. Dutta D, Puzari KC, Gogoi R, Dutta P (2014) Endophytes: Exploitation as a tool in plant protection. Braz Arch Biol Technol 57(5):621–629. https://doi.org/10.1590/S1516-89132014020043
26. Vurukonda SSKP, Giovanardi D, Stefani E (2018) Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int J Molecular Sci 19(4):952. https://doi.org/10.3390/ijms19040952
27. Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003
28. Savi DC, Aluizio R, Glienke C (2019) Brazilian plants: an unexplored source of endophytes as producers of active metabolites. Planta Med 85(08):619–636. https://doi.org/10.1055/a-0847-1532
29. Silveira Rabelo AC, Caldeira CD (2018) A review of biological and pharmacological activities of Baccharis trimera. Chem Biol Interact 296:65–75. https://doi.org/10.1016/j.cbi.2018.09.002
30. Oliveira EA (2012) O Parque Nacional Dos Campos Gerais: Processo de Criação. Universidade Federal do Paraná, Caracterização Ambiental e Proposta de Priorização de Áreas Para Regularização Fundiária
31. Paulitsch F, Klepa MS, da Silva AR et al (2019) Phylogenetic diversity of rhizobia nodulating native *Mimosa gymnas* grown in a South Brazilian ecotone. Mol Biol Rep 46(1):529–540. https://doi.org/10.1007/s11033-018-4506-z
32. Paulitsch F, Dall'Agnol RF, Delamuta JRM, Ribeiro RA, da Silva Batista JS, Hungria M (2019) *Paraburkholderia guartelaensis* sp. nov., a nitrogen-fixing species isolated from nodules of *Mimosa* gymnas in an ecotone considered as a hotspot of biodiversity in Brazil. Arch Microbiol 201(10):1435–1446. https://doi.org/10.1007/s00203-019-01714-z
33. Vieira MLA, Johann S, Hughes FM, Rosa CA, Rosa LH (2014) The diversity and antimicrobial activity of endophytic fungi associated with medicinal plant *Baccharis trimera* (*Asteraceae*) from the Brazilian savannah. Can J Microbiol 60(12):847–856. https://doi.org/10.1139/cjm-2014-0449
34. Silva FC ed. (2009) Manual de Análises Químicas de Solos, Plantas e Fertilizantes. 2nd ed. Embrapa Informação Tecnológica
35. Schulz B, Wanke U, Draeger S, Aust HJ (1993) Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycol Res 97(12):1447–1450. https://doi.org/10.1016/S0953-7562(09)80215-3
36. Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water downloaded from. Appl Environ Microbiol 49(1):1–7. https://doi.org/10.1128/aem.49.1.1-7.1985
37. Sambrook J, Russell DW (2001) Molecular cloning: A laboratory manual. 3rd ed. CSHL press
38. Weisburg WG, Barns SM, Pellettier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173(2):697–703
39. Sneath PHA, Sokal RR ed. (1973) Numerical Taxonomy. The principles and practice of numerical classification. Freeman and Company
40. Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium Series41:95–98
41. Dereeper A, Guignon V, Blanc G et al (2008) Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36(suppl_2):W465-W469. https://doi.org/10.1093/NAR/GKN180
42. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32(5):1792–1797. https://doi.org/10.1093/NAR/GKH340
43. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552. https://doi.org/10.1093/OXFORDJOURNALS.MOLBEV.A026334

44. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52(5):696–704. https://doi.org/10.1080/10635150390235520
45. Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst Biol 55(4):539–552. https://doi.org/10.1080/10635150600755453
46. Chevenet F, Brun C, Bañuls AL, Jacq B, Christen R (2006) TreeDyn: Towards dynamic graphics and annotations for analyses of trees. BMC Bioinformatics 7(1):1–9. https://doi.org/10.1186/1471-2105-7-439/FIGURES/4
47. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis Version 11. Mol Biol Evol 38(7):3022–3027. https://doi.org/10.1093/MOLBEV/MSAB120
48. Hammer Ø, Harper DAT, Ryan PD (2001) Past: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):1–9
49. Hutcheson K (1970) A test for comparing diversities based on the Shannon formula. J Theor Biol 29(1):151–154. https://doi.org/10.1016/0022-5193(70)90124-4
50. Poole RW (1974) An introduction to quantitative ecology. St. Louis, San Fan, New York
51. Magurran AE (1988) Ecological diversity and its measurement. https://doi.org/10.1007/978-94-015-7358-0
52. Christensen MJ (1996) Antifungal activity in grasses infected with *Acremonium* and *Epichloë* endophytes. Australas Plant Pathol 25(3):186–191. https://doi.org/10.1071/AP96032
53. Reis A, Boiteux LS, Henz GP (2009) Antracnose em Hortaliças da família Solanacea. Circular Técnica 79, Embrapa Horaliças
54. Reis A, Costa H, Lopes CA (2007) Epidemiologia e manejo do mofo-branco em hortaliças. Comunicado Técnico 45, Embrapa Horaliças
55. Schwyn B, Neilands JB (1987) Universal CAS assay for the detection and determination of siderophores. Anal Biochem 160:47–56. https://doi.org/10.1016/0003-2697(87)90612-9
56. Payne SM (1994) Detection, isolation, and characterization of siderophores. Methods in Enzimology. Academic Press (235):329–344. https://doi.org/10.1016/0076-6879(94)35151-1
57. Youssef N, Duncan K, Nagle D, Savage K, Knapp R, Mcinerney M (2004) Comparison of methods to detect biosurfactant production by diverse microorganism. J Microbiol Methods 56:339–347. https://doi.org/10.1016/j.mimet.2003.11.001
58. Labeda DP, Dunlap CA, Rong X et al (2016) Phylogenetic relationships in the family Streptomycetaceae using multi-locus sequence analysis. Antonie van Leeuwenhoek. 110(4):563–583. https://doi.org/10.1007/S10482-016-0824-0
59. Xia Y, DeBolt S, Dreyer J, Scott D, Williams MA (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front Plant Sci 6:490
60. Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC (2016) Current knowledge and perspectives of *Paenibacillus*: A review. Microb Cell Fact 15(1):203. https://doi.org/10.1186/s12934-016-0603-7
61. Lopes R, Tsui S, Gonçalves PJRO, de Queiroz MV (2018) A look into a multifunctional toolbox: endophytic *Bacillus* species provide broad and underexploited benefits for plants. World J Microbiol Biotechnol 34(7):94. https://doi.org/10.1007/s11274-018-2479-7
62. Miljaković D, Marinković J, Balešević-Tubić S (2020) The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. Microorganisms 8(7). https://doi.org/10.3390/microorganisms8071037
63. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ (2020) *Bacillus* species in soil as a natural resource for plant health and nutrition. J Appl Microbiol 128(6):1583–1594. https://doi.org/10.1111/jam.14506
64. Dourado MN, Neves AAC, Santos DS, Araújo WL (2015) Biotechnological and agronomic potential of endophytic pink-pigmented Methylotrophic *Methylobacterium* spp. Quinn FD ed. BioMed Res Int 909016. https://doi.org/10.1155/2015/909016
65. Aquino GS, Ventura MU, Alexandrino RP et al (2018) Plant-promoting rhizobacteria *Methylobacterium komagatae* increases crambe yields, root system and plant height. Ind Crops Prod 121:277–281. https://doi.org/10.1016/j.indcrop.2018.05.020
66. De Meyer SE, Coorevits A, Willems A (2012) *Tardiphaga robiniae* gen. nov., sp. nov., a new genus in the family Bradyrhizobiaceae isolated from *Robinia pseudoacacia* in Flanders (Belgium). Syst Appl Microbiol 35(4):205–214. https://doi.org/10.1016/j.syapm.2012.02.002
67. Safronova VI, Kuznetsova IG, Sazanova AL et al (2015) Extra-slow-growing *Tardiphaga* strains isolated from nodules of *Vavilovia formosa* (Stev.) Fed. Arch Microbiol 197(7):889–898. https://doi.org/10.1007/s00203-015-1122-3
68. Menezes Júnior IA, Matos GF, Freitas KM, Jesus EC, Rouws LFM (2019) Occurrence of diverse *Bradyrhizobium* spp. in roots and rhizospheres of two commercial Brazilian sugarcane cultivars. Brazilian J Microbiol 50(3):759–767. https://doi.org/10.1007/s42770-019-00090-6
69. Gutiérrez-Zamora ML, Martínez-Romero E (2001) Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). J Biotechnol 91(2):117–126. https://doi.org/10.1016/S0168-1656(01)00332-7
70. Yanni YG, Dazzo FB, Squartini A, Zanardo M, Zidan MI, Elsadany AEY (2016) Assessment of the natural endophytic association between *Rhizobium* and wheat and its ability to increase wheat production in the Nile delta. Plant Soil 407(1/2):367–383
71. Gao JL, Sun P, Wang XM, Lv FY, Mao XJ, Sun JG (2017) *Rhizobium wenxiniae* sp. nov., an endophytic bacterium isolated from maize root. Int J Syst Evol Microbiol. 67(8):2798–2803. https://doi.org/10.1099/ijsem.0.002025
72. López-López A, Rogel MA, Ormeño-Orrillo E, Martínez-Romero J, Martínez-Romero E (2010) *Phaseolus vulgaris* seed-borne endophytic community with novel bacterial species such as *Rhizobium endophyticum* sp. nov. Syst App Microbiol 33(6):322–327. https://doi.org/10.1016/j.syapm.2010.07.005
73. Shrivastava S, Egamberdieva D, Varma A (2015) Plant growth-promoting rhizobacteria (pgpr) and medicinal plants: the state of the art. In: Springer, Cham 1–16. https://doi.org/10.1007/978-3-319-13401-7_1
74. Dobritsa AP, Samadpour M (2016) Transfer of eleven species of the genus *Burkholderia* to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. nov. to accommodate twelve species of the genera *Burkholderia* and *Paraburkholderia*. Int J Syst Evol Microbiol 66(8):2836–2846. https://doi.org/10.1099/ijsem.0.001065
75. Elshafie HS, Camele I (2021) An overview of metabolic activity, beneficial and pathogenic aspects of *Burkholderia* Spp. Metabolites 11(5):321. https://doi.org/10.3390/METABO11050321
76. Esmaeel Q, Miotto L, Rondeau M et al (2018) *Paraburkholderia phytofirmans* PsJN-plants interaction: from perception to the induced mechanisms. Front Microbiol 9:2093
77. Panpatte DG, Jhala YK, Shelat HN, Vyas R V (2016) *Pseudomonas fluorescens*: a promising biocontrol agent and PGPR for sustainable agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity: Research Perspectives. Springer India 257–270. https://doi.org/10.1007/978-81-322-2647-5_15
78. Chaudhary DK, Khulan A, Kim J (2019) Development of a novel cultivation technique for uncultured soil bacteria. Sci Rep 9(1):1–11. https://doi.org/10.1038/s41598-019-43182-x

79. Vartoukian SR, Palmer RM, Wade WG (2010) Strategies for culture of "unculturable" bacteria. FEMS Microbiol Lett 309(1):1–7. https://doi.org/10.1111/j.1574-6968.2010.02000.x
80. Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44(4):846–849. https://doi.org/10.1099/00207713-44-4-846/CITE/REFWORKS
81. Glaeser SP, Kämpfer P (2015) Multilocus sequence analysis (MLSA) in prokaryotic taxonomy. Syst Appl Microbiol 38(4):237–245. https://doi.org/10.1016/J.SYAPM.2015.03.007

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.