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Bacterial Community Composition and Presence of Plasmids in the Endosphere- and Rhizosphere-Associated Microbiota of Sea Fig (*Carpobrotus aequilaterus*)

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Abstract: The plant microbiome is one of the most important environments for ecological interactions between bacteria that impact the plant and the ecosystem. However, studies on the diversity of mobile genetic elements (such as plasmids) associated with the plant microbiome are very scarce. Here, we determined the bacterial community composition and the occurrence of plasmids in the microbiota associated with sea fig, *Carpobrotus aequilaterus* (N.E. Br.), a succulent species widely used as an ornamental plant in Chile. The abundance and composition of the endophytic and rhizospheric bacterial communities were determined by quantitative PCR (qPCR) and DNA metabarcoding analysis. Plasmid diversity in the plant microbiome was determined by plasmid DNA extraction and screened by endpoint PCR of backbone genes for four different incompatibility groups (Inc). The results showed about 106 copies of the 16S rRNA gene in the endosphere and rhizosphere, showing significant differences according to the diversity index. Proteobacteria (Pseudomonadota; 43.4%), Actinobacteria (Actinomycetota; 25.7%), and Bacteroidetes (Bacteroidota; 17.4%) were the most dominant taxa in both plant compartments, and chemoheterotrophy (30%) was the main predicted function assigned to the microbiota. Plasmid diversity analysis showed the presence of transferable plasmids in the endosphere and rhizosphere of *C. aequilaterus*, particularly among environmental plasmids belonging to the IncP and IncN incompatibility groups.

Keywords: *Carpobrotus aequilaterus*; sea fig; Chilean pigface; endosphere; plasmids; ornamental plant; plant microbiome; rhizosphere

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1. Introduction

Over the last several decades, conjugative plasmid-mediated horizontal gene transfer (HGT) occurring between bacteria in the environment has acquired a high relevance in public health and environmental sciences [1–4]. Conjugative plasmids are mobile genetic elements (MGEs), self-replicative and auto-transferable, that usually carry genes involved in antimicrobial resistance, virulence, heavy-metal resistance, and degradation of compounds. These MGEs confer adaptative traits to the bacterial host cells [5,6]. In this context, plasmid transfer events have been reported within complex bacterial communities in a wide variety of environments, such as soils, plants, and the animal gut [7–10]. Plasmid transfer between donor and recipient cells depends on many abiotic and biotic factors, including intrinsic

plasmid incompatibility (Inc groups) [6]. The long-term prevalence of plasmids in an environment provides insight into how the bacterial community acquires new traits via HGT in the environment [11], where a single species or bacterial cell can act as a plasmid reservoir harboring one or more plasmid types [11]. In this context, niches that harbor a high diversity and richness of bacterial species might also be rich in exchangeable genes that may contribute to the competitiveness of bacteria in changing environments [12–14].

Many studies have now revealed that plants represent a complex environment harboring an enormous amount and diversity of microorganisms (also often called the plant microbiome) where plant–bacteria interactions are crucial for plant fitness and, therefore, for terrestrial ecosystem functioning [15,16]. Particularly, the rhizosphere (the soil directly influenced by plant roots) is widely recognized as one of the main hotspots of biological activity in many ecosystems. The rhizosphere often contains a great quantity and variety of nutrients for bacteria, which promote microbial activities and interactions [17], often associated with the concomitant gene exchange by conjugative plasmid events [18–20]. Recently, endophytic bacteria (those living in internal plant tissues) have also attracted the attention of microbiologists, plant physiologists, and agriculturists due to their close interactions with plant hosts, improving plant growth, stress tolerance, and disease control, particularly in agronomically relevant plant species [21]. However, since the role of bacteria as plasmid reservoirs promoting HGT in the plant microbiome is still poorly investigated and understood, major studies on plant–bacteria interactions mediated by plasmid transfer in natural vegetation or agroecosystems are required [22].

While several studies revealed plasmid transfer in compartments of model and agronomically relevant plant species [3,10,14], the presence, diversity, transfer rates, and prevalence of conjugative plasmids in the plant–soil continuum of urban areas or built environments remain an unexplored "black box" [17,22]. In this context, ornamental plants can acquire a special relevance because they are widely planted and grown in many outdoor (e.g., parks, gardens, squares, etc.) and indoor (e.g., houses, offices, hospitals, etc.) environments.

Carpobrotus aequilaterus (Haw.) N.E.Br. (Aizoaceae family, Magnoliopsida class) is a succulent plant species, whose common names include Chilean pigface, sea fig, ice plant, pigface, and angled pigface. This clonal plant species is native to South Africa and widely distributed in Australia, Mexico, the U.S.A., and Europe [23–26]. This plant was commonly introduced for ornamental use in residential areas, landscaping in coastal areas, and sand dune stabilization [23,24]. Its ability to resist drought and salt stress [23] makes this plant an aggressive invader in many coastal ecosystems [25,26], modifying soil properties such as nutrient content and water retention [24]. *C. aequilaterus* in Chile is popularly used as an ornamental plant in outdoor and indoor environments. Besides studies on its salt and drought tolerance or ornamental use, *C. aequilaterus* has also been studied in Chilean mining tailing due to its phytostabilization capacity of metals and sulfur and its abundant aerial biomass where organic matter is incorporated [27]. However, little is known about the microbiome of this urban plant species. Under this scenario, the present study was conducted to explore the composition of the bacterial community and the occurrence of plasmids in the rhizosphere and endosphere of *C. aequilaterus* collected in urban areas of Chile.

2. Materials and Methods

2.1. Plant Specimens

Specimens of *C. aequilaterus* were collected from an urbanized area in Calama city (22°27′45″ S 68°55′38″ W), located in the Atacama Desert in northern Chile. A cleaned spade was used to carefully remove intact specimens from the soil at a depth of 0–20 cm. The plants were placed in sterile polyethylene bags and transported to the Applied Microbial Ecology Laboratory (EMALAB) at Universidad de La Frontera. When the radicle emerged (after approximately 3 weeks), the specimens were planted and maintained individually in pots containing 600 g of a sand/soil substrate (3:1) under greenhouse conditions

(temperature, 21 °C; humidity, 65%; natural light); they were watered once per week to field capacity for 60 days until sampling preparation.

2.2. Sampling and DNA Extraction

Six plants were independently used to isolate endosphere tissues rhizosphere soil samples. Samples of the endosphere from each plant specimen were processed as described by Govindasamy et al. [28], with a few modifications. The aerial parts of the plants were washed and surface-sterilized by repeated immersion in 70% (vol vol^{-1}) ethanol for 3 min, followed by 2.5% (vol vol^{-1}) sodium hypochlorite (NaClO) for 5 min. The plant tissues were exhaustively rinsed with sterile distilled water (SDW). Portions of the tissues (1–2 g) were aseptically cut, placed into sterile plastic tubes, stored at −80 °C, and then used for genomic DNA (gDNA) extraction. In parallel, samples of rhizosphere soil were processed as described by Lagos et al. [29]. Soil influenced by the roots was carefully collected in sterile polypropylene microtubes. Around 1–2 g of rhizosphere samples was stored at –80 °C and then used for gDNA extraction.

Total gDNA was extracted by using the E.Z.N.A.®Bacterial DNA Kit for Soil (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Successful DNA isolation was confirmed by agarose gel electrophoresis using TAE 1× buffer, 1% agarose gels, and UV irradiation. The quality and quantity of the gDNA extracts were measured by the A280/A260 absorbance ratio using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Appropriate dilutions of gDNA extracts were stored at −80 °C and used for further molecular analyses.

2.3. Bacterial Community Analyses

2.3.1. Quantification of the Bacteria

Dilutions of gDNA were used to estimate the abundance of bacteria (16S rRNA gene) in each plant compartment by quantitative PCR (qPCR) using the universal primer sets Bac1369F (5′–CGG TGA ATA CGT TCY CGG–3′) and Prok1492R (5′–GGW TAC CTT GTT ACG ACT–3′) with a StepOne Real-Time PCR System (ThermoFisher Scientific, Inc., Waltham, MA, USA) and PowerUpTM SYBRTM Green Master Mix (Applied Biosystems™, Foster City, CA, USA), using ~25 ng μL^{-1} of gDNA as described by Jorquera et al. [30]. The copy number of the 16S rRNA gene was calculated using *Escherichia coli* standards, built with dsDNA gBlock® Gene Fragments (Integrated DNA Technologies, Inc., Coralville, IA, USA), and the equation [concentration of the dsDNA gBlock® Gene Fragment in ng μL^{-1}] × [molecular weight in fmol ng^{-1}] × [Avogadro's number] = copy number, following the method described by Whelan et al. [31]. Based on the standard curves, the absolute quantification (AQ) of the 16S rRNA gene was expressed as copy number per gram of dry soil or tissue (gene copy g^{-1} of soil or tissue) and later used to determine the relative quantification (RQ) of oriT genes in relation to the 16S rRNA gene, as described by Acuña et al. [32].

2.3.2. DNA Metabarcoding Analysis

The diversity, structure, and putative functions of the bacterial community in both plant compartments were investigated by using DNA metabarcoding analysis. The V4 region of the 16S rRNA gene was amplified by PCR using the primers 341F (5′–CCT ACG GGN GGC WGC AG–3′) and 805R (5′–GAC TAC HVG GGT ATC TAA TCC–3′) [33]. The PCR reactions were carried out with reagents supplied with GoTaq® Flexi DNA Polymerase (Promega Co., Madison, WI, USA) as follows: the denaturalization was performed at 95 °C for 10 min, the annealing temperature was initially set at 65 °C and then decreased by 0.5 °C every cycle until reaching 55 °C, a value that was maintained for 1 min, and the extension was performed at 72 °C for 3 min. The 16S rRNA gene libraries were gel-purified using the AxyPrep DNA Gel Extraction Kit (Axygen®, Corning, NY, USA), and library integrity was confirmed by the 4150 TapeStation System (Agilent, Santa Clara, CA, USA). Sequencing was conducted using barcode primers and the dual indexing method. The DNA fragments were pooled and paired-end sequenced on the Illumina MiSeq platform (Illumina, Inc., San

Diego, CA, USA) at the Scientific and Technological Bioresources Nucleus of Universidad de La Frontera (BIOREN–UFRO), Temuco, Chile.

2.3.3. DNA Data Analyses

The data were analyzed using R (version 4.2.3). Firstly, QIIME 2 (Version 1.9.1) artifacts were loaded with the R package qiime2R into the R software and stored as a phyloseq-class object. The sequence data were rarefied to a sampling depth of 11,000 per sample. Chimeras were screened and removed with USEARCH using the UCHIME algorithm. Chimeric-free sequences were grouped into operational taxonomic units (OTUs) at 97% similarity by using UPARSE (version 7.0.1090) [34]. Further analyses were performed with the R package phyloseq (citation: R, qiime2R, phyloseq) for visualization and general data handling. OTUs with low prevalence (singletons) were filtered out (abundance <10 reads or prevalence <5% in all samples). Taxonomy was assigned using the DADA2 package (Version 1.16.0) [35], using the Silva taxonomy database (silva-132–99–nb–classifier.qza) and removing sequences identified as mitochondrial and chloroplast. For normalization, the feature table data (OTU counts) were transformed into relative abundance by dividing them by the total reads of each sample (total-sum scaling) and multiplying them by 1000 to achieve integer proportions. Taxonomy summaries were accomplished by merging OTUs of the same taxonomic rank and calculating their relative abundance in the samples. Richness (OTUs observed, Chao1) and alpha diversity indices (Shannon) were estimated within the phyloseq package [36]. Beta diversity was determined by calculating the Bray–Curtis dissimilarity between all samples and visualized in a non-metric multidimensional scaling (nMDS) plot with the R package vegan [37]. Global statistical significance of the differences between sample types was assessed by a permutational multivariate analysis of variance (PERMANOVA) using 999 permutations. The most correlated ($R2 > 0.6$) OTUs were added to the nMDS ordination plot. Finally, to explore the functional trait prediction of the bacterial communities, the functional annotation of prokaryotic taxa (FAPROTAX project) [38] database and scripts were used, assigning environmental functions as described by Zhang et al. [39].

2.4. Occurrence of Plasmids

2.4.1. Detection of Plasmids

The occurrence of conjugative plasmids in the *C. aequilaterus*-associated microbiota was determined by endpoint PCR as follows. First, plasmid DNA (pDNA) was extracted from endosphere and rhizosphere samples by using the QIAGEN Plasmid Mini Kit (QIAGEN, Hilden, Germany) according to methods described by Zhang et al. [40] and following the protocol of the manufacturer. Specific plasmid primer sets described by Gotz et al. [41] and Carattoli et al. [42] were used (Table 1) to amplify the pDNA fragments from genes of incompatibility (Inc) groups contained in plasmid "backbone" regions involved in the replication (IncP, trfA1/2, and korA; IncN, rep; IncW, oriV; and IncQ, oriV, and repB) and transfer (IncP, oriT, and traG; IncN, oriT; and IncW, oriT, and trwAB) of plasmids. The PCR reactions contained 1× PCR buffer, 1mM deoxynucleoside triphosphates (dNTPs), $MgCl_2$ 1.5 mM, 5U u Promega GoTaq® DNA polymerase (Promega Co., Madison, WI, USA), 5 ng μL^{-1} of pDNA, 0.5 mM solutions of each primer set, and nuclease-free water. Thirty-five cycles of amplification were performed, each consisting of 1 min of denaturation at 94 °C, 1 min of primer annealing at the annealing temperature as described in Table 1, and 1 min of primer extension at 72 °C, followed by a final extension step of 10 min at 72 °C. The PCR products were visualized under UV light following electrophoresis.

Table 1. Primer sets used for PCR amplification in endosphere and rhizosphere samples of *C. aequilaterus* according to the different compatibility (Inc) groups.

Inc Group	Region	Function	Primer Sequence (5′-3′)	Annealing Temperature (°C)
IncN	*rep 1*	Replication	AGT TCA CCA CCT ACT CGC TCC G	55
	rep 2	Replication	CAA GTT CTT CTG TTG GGA TTC CG	
	oriT1	Transfer	TTG GGC TTC ATA GTA CCC	49
	oriT2	Transfer	GTG TGA TAG CGT GAT TTA TGC	
IncP	*oriT1*	Transfer	CAG CCT CGC AGA GCA GGA T	57
	oriT2	Transfer	CAG CCG GGC AGG ATA GGT GAA GT	
	trfA1 1	Replication	ATG ACG ACC AAG AAG CG	57
	trfA1 2	Replication	AAC CCC CAG CCG GAA CTG	
	traG 1	Transfer	CTG CGT CAC GAT GAA CAG GCT TAC C	63
	traG 2	Transfer	ACT TCC AGC GGC GTC TAT GTG G	
IncQ	*repB 1*	Replication	TCG TGG TCG CGT TCA AGG TAC G	62
	repB2	Replication	CTG TAA GTC GAT GAT CTG GGC GTT	
IncW	*oriV 1*	Replication	GAC CCG GAA AAC CAA AAA TA	58
	oriV2	Replication	GTG AGG GTG AGG GTG CTA TC	
	oriT 1	Transfer	TCT GCA TCA TTG TAG CAC C	51
	oriT2	Transfer	CCG TAG TGT TAC TGT AGT GG	

2.4.2. Quantification of IncP Plasmids

Since the endpoint PCR results indicated the occurrence of IncP plasmids in both plant compartments, the abundance of IncP plasmids was determined by qPCR using 5 ng μL^{-1} of pDNA and the primer set *oriT1* (5′CAG CCT CGC AGA GCA GGA T 3′) and *oriT2* (5′CAG CCG GGC AGG ATA GGT GAA GT 3′). The PCR conditions were as follows: enzyme activation step at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min of annealing plus extension at 60 °C. The PCR reactions were performed in triplicate with 20 $\mu g\ L^{-1}$ of total plasmid DNA in a StepOne Plus™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) and following the manufacturer's instructions. The abundance of IncP plasmids was estimated based on a standard curve built using pDNA from a derivative of the broad-host-range plasmid RK2 (IncP) [43].

2.5. Statistical Analysis

Assumption tests for normality (Shapiro–Wilk test) and equality of variance (Levene's test) of data were initially performed. Statistical significance was calculated with either the two-sample *t*-test (normally distributed data with equal variances) or the Wilcoxon Rank Sum Exact Test (data with equal variances).

3. Results

3.1. Bacterial Community

The qPCR analysis revealed that the abundance of bacteria ranged from 6.9×10^5 to 1.5×10^6 copies of 16S rRNA gene g^{-1} of endosphere tissue and from 6.2×10^5 to 2.2×10^6 copies of 16S rRNA gene g^{-1} of rhizosphere soil (Figure 1A). Significant differences in copy number between the two plant compartments were not observed ($p = 0.4894$). In contrast, the alpha diversity analysis revealed significantly ($p = 0.0005$) greater richness in the endosphere samples compared with the rhizosphere samples, ranging from 440 to 568 of the observed OTUs and from 296 to 437 of the observed OTUs, respectively (Figure 1B). Similarly, a higher diversity was also observed in the endosphere samples than in the rhizosphere samples, as revealed by the Shannon index, with values from 5.8 to 6.1 and from 5.3 to 5.8, respectively.

Statistical differences were observed in the Shannon ($p = 0.0016$) and Chao1 ($p = 0.0011$) indices between samples obtained from the endosphere and rhizosphere.

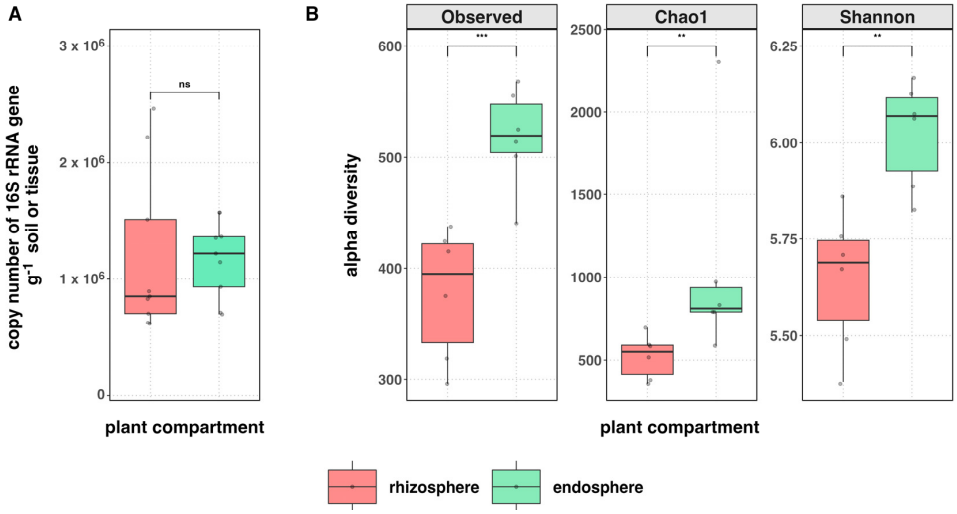

Figure 1. Abundance (**A**) and alpha diversity (**B**) of the bacterial community in the endosphere and rhizosphere of *C. aequilaterus*. Legend: ns, non-significant differences; ** denotes significant differences at $p < 0.01$ ($n = 3$); *** denotes significant differences at $p < 0.001$ ($n = 3$).

Differences between endophytic and rhizosphere bacterial communities were also revealed by beta diversity analyses (Figure 2). The nMDS analysis showed the presence of two clusters at 95% significance. In addition, the bacterial structure in the endosphere samples was mainly composed of members of the orders Myxococcales, Sapropirales, Gemmatimonadales, and Xanthomonadales, whereas the rhizosphere comprised two groups, one characterized by the Actinomycetales and Myxococcales orders, and the other by the Vibrionales and Rhodobacterales orders (Figure 2).

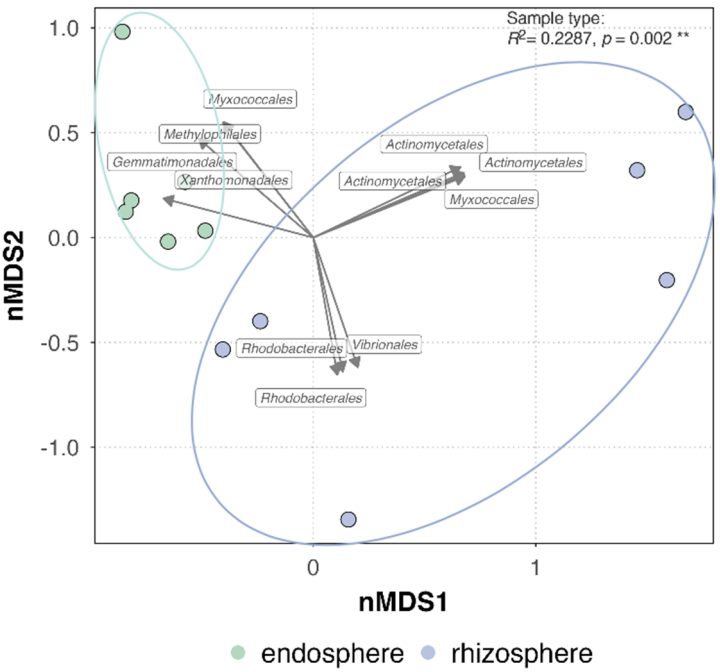

Figure 2. Nonmetric multidimensional scaling (nMDS) analysis of the bacterial community found in the endosphere and rhizosphere of *C. aequilaterus*. The most abundant bacterial orders are highlighted. ** $p < 0.01$.

Taxonomic analyses of the bacterial community revealed that the phylum Proteobacteria showed a high relative abundance in the endosphere (43.4%) and rhizosphere (39.7%), followed by the Actinobacteria (25.7%) in the rhizosphere and the Bacteroidetes in the endosphere (17.4%) (Figure 3A). Other abundant phyla found in the endosphere and rhizosphere samples were the Acidobacteria (5.4% and 4.6%, respectively), Gemmatimonadetes (5.1% and 2.3%, respectively), and Verrumicrobia (3.6% and 1.9%, respectively). Moreover, assignments at the order level (Figure 3B) indicated that members of the Actinomycetales order were the most abundant (21.3%) in the rhizosphere samples, followed by members of the Xanthomonadales (5.5%), Burkholderiales (5%), and Sphingomonadales (4.8%) orders. In the endosphere samples, members of the Xanthomonadales order were the most abundant (11.4%), followed by members of the Burkholderiales (7%), Actinomycetales (6.9%), and Sphingomonadales (6.8%) orders.

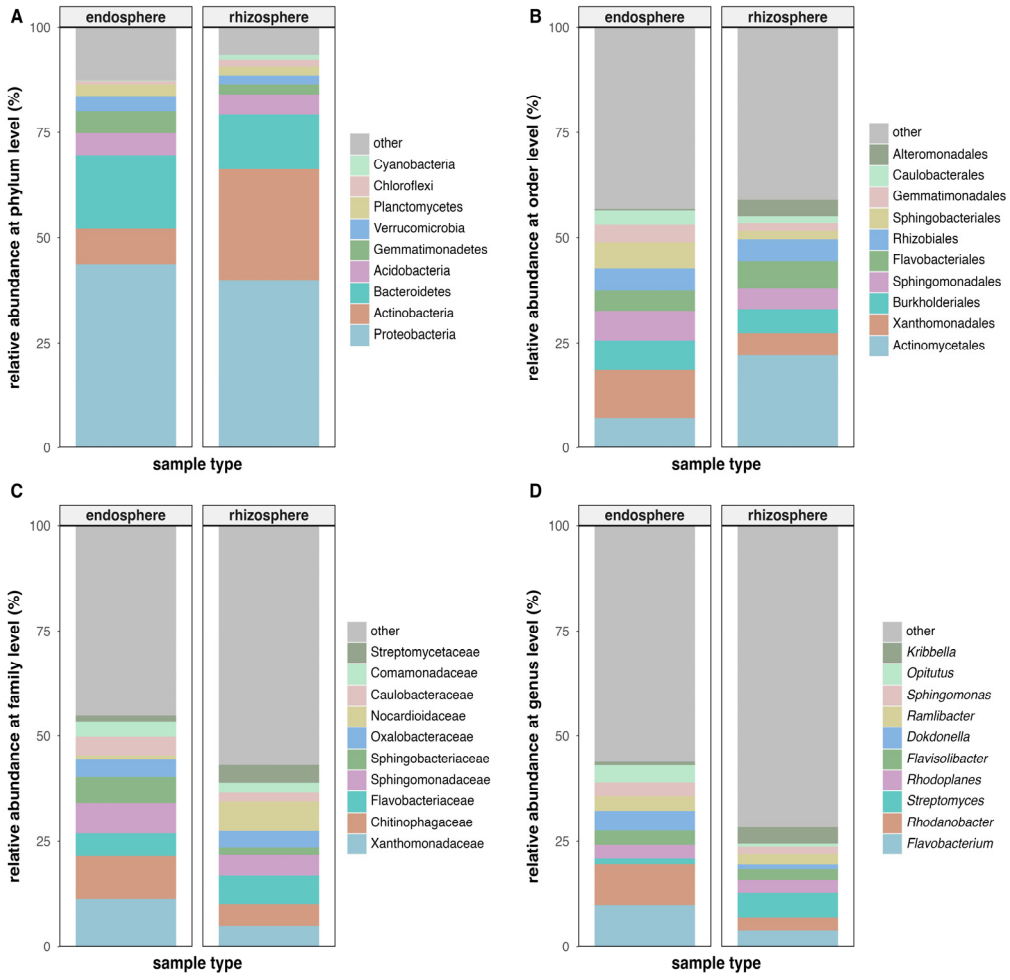

Figure 3. Mean (n = 3) relative abundance of the taxonomic structure of the bacterial community in the endosphere and rhizosphere of *C. aequilaterus* at the phylum (**A**), order (**B**), family (**C**), and genus (**D**) levels.

Family-level analyses showed higher relative abundances of members of the Xanthomonadaceae (11%), Chitinophagaceae (10%), and Sphingomonadaceae (7%) families in the endosphere samples, whereas Flavobacteriaceae (6.8%) and Nocardioidaceae (6.7%) showed the higher relative abundances in the rhizosphere samples (Figure 3C). Lastly, genus-level analyses showed greater relative abundances of the genera *Flavobacterium* (9.6%) and *Rhodanobacter* (Xanthomonadales) (9.5%) in the endosphere samples, whereas members of the genus *Streptomyces* (5.8%) had the greatest relative abundance in the rhizosphere samples (Figure 3D). However, statistical differences ($p < 0.05$) between the

endosphere and the rhizosphere microbiota were only found for the genera *Dokdonella*, *Sphingomonas*, and *Opitutus* (Supplementary Table S1).

Despite these taxonomic differences, functionality appeared conserved, and the FAPROTAX analysis showed chemoheterotrophy and aerobic chemoheterotrophy as the major functions assigned to the microbiota in both studied plant compartments, with values equal to or higher than 30% (Figure 4). Overall, similar functions were attributed to the microbiota in the two plant compartments, particularly, functions related to fermentation and nitrogen cycling, such as nitrate reduction, nitrate respiration, nitrogen respiration, nitrate denitrification, and nitrite denitrification, among others. Interestingly, greater relative abundances of sequences were found for microorganisms involved in the degradation of aromatic compounds and in oxygenic photoautotrophy and for photosynthetic cyanobacteria in the rhizosphere compared with the endosphere. In contrast, higher abundances of sequences were found for microorganisms involved in methylotrophy and methanol oxidation in the endosphere compared with the rhizosphere.

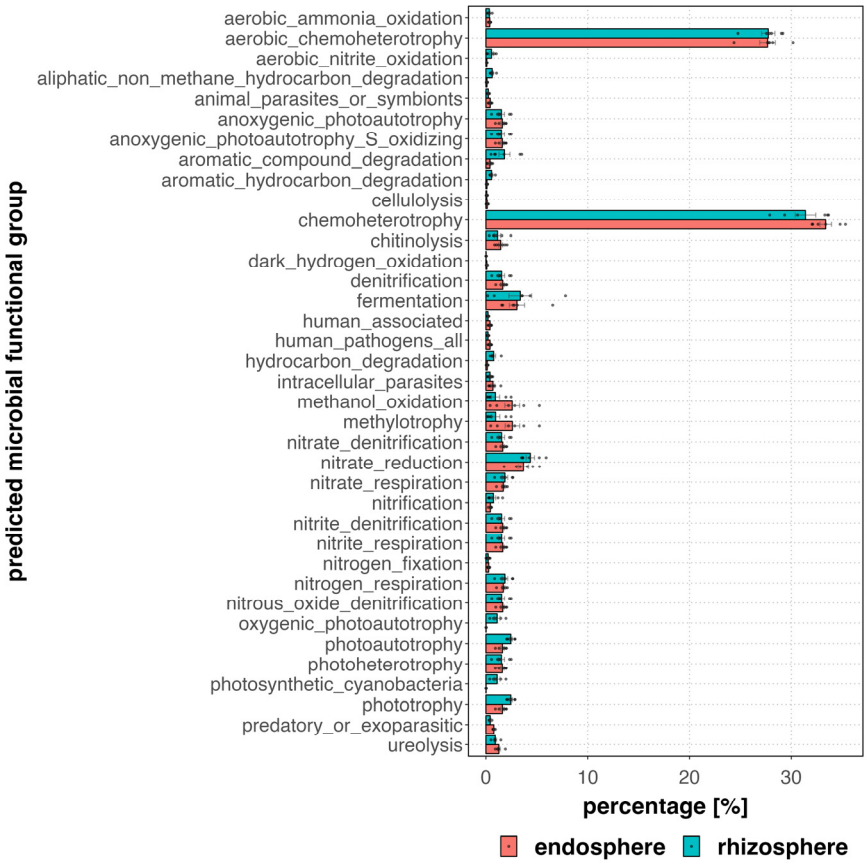

Figure 4. Mean (n = 3) relative abundances of predicted microbial functional groups of the bacterial community in the endosphere and rhizosphere of *C. aequilaterus*. Dots represent outlier values and bars represent the standard deviation.

3.2. Occurrence of Plasmids

The use of specific endpoint PCR primer sets to identify genes involved in the replication and transfer of plasmids resulted in positive amplifications in both endosphere and rhizosphere samples. In the endosphere samples, amplicons were observed with the use of primer sets for the trfA1 (IncP) and oriT (IncN and IncP) genes, while amplicons for the oriV (IncW) and oriT (IncN and IncP) genes were observed in the rhizosphere samples (Table 2). In contrast to what observed for the endosphere, signals for the rep (IncN) and oriV (IncW) genes were only revealed in the rhizosphere by PCR.

Table 2. PCR amplification in endosphere and rhizosphere samples of *C. aequilaterus* revealed by specific primer sets for plasmids in different compatibility (Inc) groups.

		Replication				Transference			
		IncN	IncP	IncQ	IncW	IncN	IncP		IncW
Sample		rep	trfA1	repB	oriV	oriT	oriT	traG	oriT
Endosphere	E1.1	–	+	–	–	+	+	(+)	–
	E1.2	–	+	–	–	+	+	(+)	–
	E1.3	–	+	–	–	+	+	(+)	–
	E2.1	–	+	–	–	+	+	(+)	–
	E2.2	–	+	–	–	+	+	(+)	–
	E2.3	–	+	–	–	+	+	(+)	–
	E3.1	–	+	–	–	+	+	(+)	–
	E3.2	–	+	–	–	+	+	(+)	–
	E3.3	–	+	–	–	+	+	(+)	–
Rhizosphere	R1.1	+	(+)	(+)	+	+	+	(+)	(+)
	R1.2	+	(+)	(+)	+	+	+	(+)	(+)
	R1.3	+	(+)	(+)	+	+	+	(+)	(+)
	R2.1	+	(+)	(+)	+	+	+	(+)	(+)
	R2.2	+	(+)	(+)	+	+	+	(+)	(+)
	R2.3	+	(+)	(+)	+	+	+	(+)	(+)
	R3.1	+	(+)	(+)	+	+	+	(+)	(+)
	R3.2	+	(+)	(+)	+	+	+	(+)	(+)
	R3.3	+	(+)	(+)	+	+	+	(+)	(+)

+: positive amplification; (+): weak signal of amplification; –: negative amplification.

We also specifically examined the abundance of IncP plasmids in both studied plant compartments by using a primer set for the *oriT* gene. The qPCR analysis showed from 0.49 to 12 × 10^2 copies of the *oriT* gene g^{-1} of endosphere tissue and from 1.2 to 8.9 × 10^2 copies of the *oriT* gene g^{-1} of rhizosphere soil (Figure 5). No statistical difference was found between the plant compartments ($p = 0.175$).

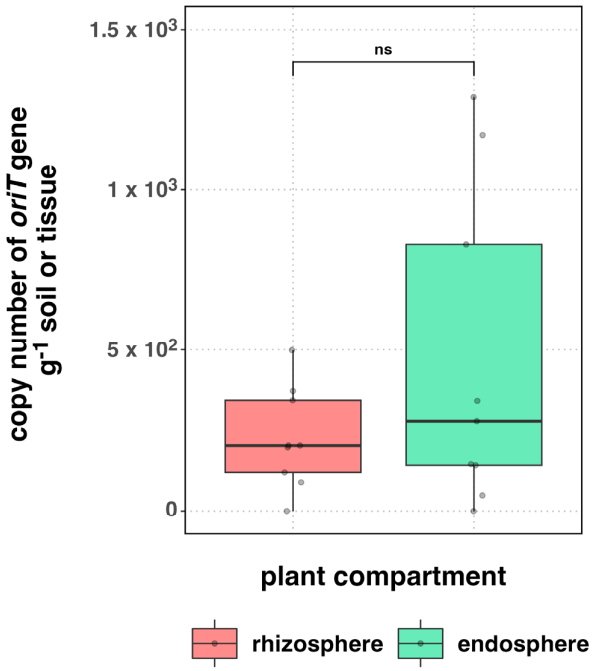

Figure 5. Quantification of IncP plasmids in the endosphere and rhizosphere of *C. aequilaterus* by quantitative PCR. Legend: ns, non-significant differences ($p < 0.05$; two-sample *t*-test). Dots represent outlier values and bars represent the standard deviation.

4. Discussion

4.1. Bacterial Communities

Here, we investigated the rhizospheric and endophytic bacterial communities, as well the plasmids in the *C. aequilaterus* (sea fig) microbiome. Our analysis of the bacterial communities showed that there were from 10^5 to 10^6 copies of the 16S rRNA gene g^{-1} of rhizosphere soil or endosphere tissue of *C. aequilaterus*. The endosphere values found here are lower than those observed for plants grown in desert ecosystems. Particularly, Zhang et al. [44] reported values ranging from 10^{10} to 10^{12} in the endosphere of *Distichlis spicata* and *Pluchea absinthioides* plants growing in the Atacama Desert of northern Chile. Similarly, greater values (from 10^7 to 10^{10} copies of the 16S rRNA gene g^{-1} of soil) were also reported in the rhizosphere of native plants (*Atriplex* spp.) from the Atacama Desert by Acuña et al. [32]. It appears that the 16S rRNA copy number is not consistent and likely varies by plant species and location. For example, Li et al. [45] reported similar values to ours in the rhizosphere of two species of *Haloxylon* growing in the desert and sand dunes, with about 10^6 copies of the 16S rRNA genes g^{-1} of soil. In this context, it was also indicated that limited nutrient and water in soils can influence the microbiome recruitment by plants, leading to the selection of specific soil bacterial communities with beneficial functions that might help plants under harsh environmental conditions [46,47]. Thus, plant roots can modulate positive and negative bacterial interactions, as observed across the altitudinal vegetation belt in the Atacama Desert by Mandakovic et al. [48]. Similarly, studies on two cactus species, *Myrtillocactus geometrizans* and *Opuntia robusta*, in arid ecosystems revealed that plant compartments, plant species, site, and season played a crucial role in the assembly of the microbiota [49].

Sequence analysis revealed that OTUs (440 to 568) and Shannon indices (5.8 to 6.1) were significantly higher for the endosphere of the examined plant than for the endosphere of plants from the Atacama Desert, determined by Zhang et al. [44], which ranged from 148 to 245 and from 2.28 to 4.41, respectively. Additionally, it is necessary to mention that *C. aequilaterus* is a succulent plant considered to be one of the most remarkable examples of convergent evolution, particularly because of the presence of large cells for water storage (90 to 95%) in the leaf endosphere [50]. The water content and particular endospheric characteristics of *C. aequilaterus* might regulate and promote higher richness and diversity compared with the rhizosphere in desert environments, as observed in our study.

Despite these results, our study also showed that the Shannon index values were significantly ($p < 0.01$) lower in the rhizosphere (5.3 to 5.8) compared with the endosphere and were much higher than those reported by Gao et al. [51], who observed values from 2.8 to 4.0 for the rhizosphere bacterial communities of the shrub *Caragana microphylla* grown in arid and semiarid environments. It should be noted, however, that while our results differed from those of other studies reporting that the rhizosphere harbors a major richness and diversity compared to the endosphere [39,52,53], some studies also highlighted that the endophytic bacteria of plants growing in dry environments represent an important niche of vital microbial functions for plant development, such as ACC deaminase production, nitrogen fixation, phosphorus solubilization, root biomass, among others [28,49,54]. Studies also revealed that endophytic characteristics are relevant for cactus seed germination and for the survival of plants growing in arid ecosystems [49,55]. Accordingly, the presence of a great diversity of microbes in the endosphere may be just as important as it is in the rhizosphere for plant growth under a variety of limiting conditions.

Our taxonomic analyses indicated that the phyla Proteobacteria (Pseudomonadota; from 39 to 43%), Actinobacteria (Actinomycetota; from 8 to 25%), and Bacteroidetes (Bacteroidota; from 13 to 17%) were the most abundant microbiota components in both studied compartments. Members of these phyla were observed as dominant taxa in the endosphere and rhizosphere of a great variety of plant species [24,56,57], including succulent plants such as *Carpobrotus edulis*, taxonomically related to sea fig, which showed relative abundances from 48 to 38% and from 29 to 27% for Proteobacteria and Actinobacteria in rhizosphere samples, respectively [24,58]. Moreover, Rodriguez-Caballero et al. [59] reported

that Actinobacteria (43%) and Proteobacteria (42%) were dominant taxa in the rhizosphere of *C. edulis*. At the family level, members of the Sphingomonadaceae, Nocardioidaceae, Micrococcaceae, Chitinophagaceae, and Rhizobiaceae families were reported as the most representative in the *C. edulis* rhizosphere [58–60]. While our results similarly showed that the Chitinophagaceae and Sphingomonadaceae families were the most abundant taxa in the rhizosphere of *C. aequilaterus*, we also found that members of the families Flavobacteriaceae and Xanthomonadaceae were abundant.

Based on the FAPROTAX analysis, chemoheterotrophy and aerobic chemoheterotrophy were the most assigned predicted functions in the bacterial community, followed by functions involved in fermentation and nitrogen cycling (e.g., nitrate reduction). These results agree with several studies on plant–soil microbiomes, reporting that chemoheterotrophs are commonly observed and isolated from compartments of plants grown in arid ecosystems [56,61,62]. Similarly, according to Novoa et al. [24] and Rodriguez-Caballero et al. [59], *Carpobrotus* increases the soil organic content due to the decomposition of its recalcitrance tissue. This deposition may allow plant to selectively recruit bacteria taxa with specific functions, such as the degradation of organic compounds as a source of energy [24,59]. In agreement with this, we reported in both plant compartments the genus *Flavobacterium*, which is involved in nitrogen cycling and is a facultative anaerobic chemoheterotroph that uses organic compounds as electron donors and a source of cellular carbon [63].

Interestingly, aromatic compound degradation was another microbial function predicted to be present in the rhizosphere of *C. aequilaterus*. According to the taxonomic analysis, members of the family Sphingomonadaceae are commonly found in soil ecosystems and carry out a variety of metabolic processes such as the catabolism of various organic compounds, including aromatic compounds, e.g., chlorinated phenols, and xenobiotics. Moreover, some strains of *Sphingobium* spp. were shown to detoxify phenolic contaminants and pesticides (e.g., fenpropathrin, deltamethrin, fenvalerate, cypermethrin, and permethrin), suggesting that these microbes may be useful in treating sites with pesticide waste [64,65].

4.2. Occurrence of Plasmids

Our results also showed the occurrence of environmental plasmids in microbes present in both studied plant compartments. The presence of environmental plasmids was detected in the rhizosphere, endosphere, and phyllosphere of a variety of plants, such as wheat [66], pea [67], alfalfa [68], and barley [69], among others. In the Chilean terrestrial ecosystem, to our knowledge, studies on native plasmids in plant compartments are very limited. In this sense, Jorquera et al. [70] determined the occurrence and stability of plasmids encoding for Al tolerance in the rhizospheres of pasture and crop plants growing in acidic volcanic soils in southern Chile. Using the biparental mating assay and Al as a selective pressure, this study demonstrated the occurrence of Al tolerance and antibiotic resistance plasmids in bacterial populations in the rhizosphere. Interestingly, this study also revealed that independently of the presence of Al pressure, the plasmid pRPA21 was highly stable, whereas the plasmid pOPA21 was only stable in the presence of Al pressure. This study suggests that the presence of Al tolerance plasmids might play an important role in the adaptation of bacterial communities to Al toxicity in volcanic soils.

Of the plasmids examined, we observed positive PCR reactions for IncP and IncN plasmids in the endosphere and rhizosphere samples of *C. aequilaterus*. The genes encoded by the IncP and IncN plasmids are often associated with resistance to a wide variety of antibiotics and with tolerance to heavy metals and quaternary ammonium compounds used as disinfectants [6]. IncP and IncN plasmids were also observed in plant and human pathogenic strains with multiple antibiotic resistance traits and in some strains known to harbor pesticide-degrading genes encoded by plasmids [71–73]. Marcoleta et al. [74] also reported that antibiotic resistance genes found in desert ecosystems, such as the Antarctic soil, can be encoded in plasmids. Several studies demonstrated that plants can recruit plant- and compartment-specific bacterial communities, which can significantly

differ in structure and activity depending on the plant compartment [39]. In each plant compartment, bacterial recruitment can be influenced and regulated by a wide battery of factors, including plant genotype, phenological stage, soil properties, soil fertilization, and environmental factors, among others [75]. Therefore, differentiation among bacterial communities could also influence the occurrence and/or copy number of plasmids in the endosphere and rhizosphere of *C. aequilaterus*. Another involved factor could be the incompatibility between environmental plasmids and the regulation of their prevalence and dissemination by the activity of each taxonomic group. However, major studies are still required to confirm plasmid differentiation in plant compartments of *C. aequilaterus*.

5. Conclusions

In this study, we found that *C. aequilaterus* harbors diverse bacteria living in the endosphere and rhizosphere. Our results also revealed a higher richness and diversity of the microbiota in the endosphere than that in the rhizosphere. While the total amount of 16S rRNA was not significantly different between the two compartments, a remarkable difference was observed in the alpha and beta diversity analysis, suggesting the relevance of the *C. aequilaterus* endosphere as an important plant niche. While previous studies showed that the plant rhizosphere is important for plant growth and survival in a variety of environments, our data suggest that the endosphere is likely equally important for plant growth. Our results also showed an important signal for *oriT* genes coding for transferable plasmids belonging to the IncN and IncP groups in both studied plant compartments. This study presents novel insights into the bacterial community associated with *C. aequilaterus* in urban environments. Thus, these ornamental plants might act as an important and unexplored reservoir and hotspot for the dissemination and exchange of bacterial genes with potential relevance for public health and environmental sciences.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15111156/s1. Table S1. Differences in the dominant taxa between endosphere and rhizosphere samples from *C. aequilaterus*.

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