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RHIZOBACTERIAL COMMUNITIES ASSOCIATED WITH THE FLOWERING DESERT PHENOMENON AND THEIR POTENTIAL AS PLANT-GROWTH PROMOTING BACTERIA

DOCTORAL THESIS IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF SCIENCES IN NATURAL RESOURCES

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“Rhizobacterial communities associated with the flowering desert phenomenon and their potential as plant-growth promoting bacteria.”

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THESIS OUTLINE

One of the main consequences of human activity is the acceleration of climate change. This phenomenon has caused climatic variations worldwide, mainly affecting countries and regions whose economy depends on food production. In this sense, using technologies that favor production maximizing the use of scarce resources, such as water or certain soil nutrients, becomes a real need. In this context, the selection of rhizosphere bacteria with the ability to promote plant growth and its subsequent use, by itself, or bacterial consortia, in cropping systems is an attractive alternative currently being used in agriculture.

Under climate change scenario, an increase of abiotic stresses is expected in crops. In addition to the use of bacteria isolated from conventional crops, it has also been suggested the selection of bacteria from plants grown in extreme environments, such as bacteria from rhizosphere in plants during the Flowering Desert phenomenon, occurring unevenly in the Atacama Desert, would generate an input with improved adaptive capacities to stressful conditions. This would be explained by the fact that bacteria, or bacterial consortia, from extreme environments, already have adaptations to this type of environment and, when used in horticultural production, they would contribute to mitigating the impact of the climate on production. In this Doctoral Thesis, we address the effect of a bacterial consortia, formulated from rhizobacteria of a native and representative plant from the flowering desert (*Cisthante longiscapa*) hypothesizing that "The application of rhizobacterial consortia from arid environments, such as flowering desert, will improve the growth of plants under stress by water scarcity."

In **Chapter I**, general introduction, hypothesis, general and specific objectives are presented. The general objective of this Doctoral Thesis was to evaluate the influence

of the rhizobacterial communities associated with flowering desert on the growth of plants under water scarcity stress.

Chapter II corresponds to the published manuscript titled "Composition, predicted functions and co-occurrence networks of rhizobacterial communities impacting flowering desert events in the Atacama Desert, Chile". In this chapter Flowering Desert (FD) phenomenon, categorized as the driest desert in the world, is analyzed by the rapid and infrequently blooming of a great diversity of plants, in a short period of time. While ephemeral plants are an integral part of the desert ecosystem, there are little knowledge of plant-microbe interactions that occur during FD events. Consequently, the overall goals of this present study were to investigate changes in the composition and potential functions of rhizobacterial community of *Cistanthe longiscapa* (Montiaceae) during the 2014 and 2015 FD events, and determine the composition, potential functions, and co-occurrence networks of rhizobacterial community associated with the rhizosphere of *C. longiscapa* during pre- (PF) and full flowering (FF) phenological stages. Overall, greater microbial richness and diversity were observed in rhizosphere soils during different FD events, similar to predicted functional analyses. Despite the lack of significant differences in diversity among PF and FF stages, the combined analysis of rhizobacterial community data, along with data concerning rhizosphere soil properties, evidenced differences among both phenological stages and suggested that sodium ions are relevant abiotic factors shaping the rhizosphere. Co-occurrence analysis revealed the complex rhizobacterial interactions that occur in *C. longiscapa* during FD events. This study shows that the composition and function of rhizobacteria vary among and during FD events, and some specific groups may influence the growth and flowering of native plants in the Atacama Desert.

Chapter III, titled as the submitted article "Rhizobacteria isolated during 'Flowering Desert' contribute to the mitigation of water scarcity stress in tomato plantlets", aim to investigate the effect of native plant growth-promoting rhizobacteria (PGPR), in tomato (*Solanum lycopersicum* L.) plantlets, used as model plant. As PGPR have been proposed as inoculants to mitigate abiotic stresses in plants, we isolated rhizobacteria from *Cistanthe longiscapa*, and validated them as PGPR in tomato seedlings under water scarcity conditions. First, PGPR, were isolated, characterized, and strains with one, or more plant growth-promoting traits were selected. Three PGPR consortia were formulated isolated *Bacillus* strains and then applied in tomato seedlings exposed to different periods of no irrigations, before transplanting them into definitive conditions. In general, tomato seedlings inoculated with PGPR consortia resulted in significantly ($P \leq 0.05$) greater growth and recovery rates of plantlets (88 to 100%) than those without inoculation (37 to 51 cm of height; 146 to 197 g of fresh weight; 54 to 92% of recovery) exposed to different intervals (24, 72 and 120 h) without irrigation before transplantation. Our results reveal that formulated PGPR consortia from FD are able to improve the performance of plantlets subjected to water scarcity, which can represent an alternative to farmers facing the drought events associated with water scarcity which is one of the most important effects of climate change in agriculture of semiarid and arid regions worldwide.

Finally, **Chapter IV** corresponds to general discussion, conclusions, and future directions.

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CHAPTER I

General introduction, hypothesis, and objectives

1.1 GENERAL INTRODUCTION

Climate change is a current global concern on weather modification with respect to a local or regional historical database. Such change is produced in many different time scales and consider all climatic parameters: temperature, precipitation, cloudiness, etc. These changes can be due to natural causes, but humans are the main agents of change and are likely to remain so for the next few centuries (Doran & Zimmerman, 2009; Trenberth et al., 2018). In general, it is believed that agricultural production will be affected mainly by the severity and pace of climate change and not by gradual climate trends (Lobell & Gourdji, 2012). If the change is gradual, biota will have enough time to adapt. However, if climate change is serious, it could threaten agriculture in many countries, especially those who have already suffered from changes in weather conditions, since they have less time for optimum natural selection and adaptation (Arora, 2019)

Climate change is also one of the main concerns in modern agriculture and food safety, particularly in hot arid zones where agriculture yields can be severely affected by extended periods of drought, elevated soil salinity, high-temperature oscillations, and strong UV-radiation. Extreme warm, or cold, waves and prolonged drought or intense rain periods, as a product of climatic change, have produced lower crop productions and economic losses around the globe (Emanuel et al., 1985; Peterson et al., 2013; Qiao et al., 2017). To reduce the impact of unexpected climatic events, the use of alternative technology is highly required to maintain the current production levels in agriculture affected negatively by climate change around the globe.

In our country, this phenomenon is reflected in an increase in maximum and minimum temperatures recorded in the last 50 years, as well as reductions of water irrigation supply (Roco et al., 2014; Fernandez et al., 2019). Increasing temperatures

combined with rainfall deficit affect, mostly, agricultural lands located between the north-central and south-central areas of Chile.

In Figure 1, the Standardized Precipitation Index in October 2015, is shown. With this index it is possible to quantify and compare the intensities of rainfall deficits between areas with very different climates considering different time ranges (Minagri, 2015). In this sense, the agricultural boundaries of Chile will be modified; there will be crops in the central area of the country that can only be developed further south as is possible to appreciate now (ODEPA, 2013). An example of this new scenario, according to data from INE, is the amount of land dedicated to vegetable crops. Corn, lettuce, and tomato are the most important vegetables for fresh consumption, but even as production has increased, the area planted has declined (from Coquimbo to Maule 70,000 ha in 2014 to 64,640 ha in 2020) and has become increasingly concentrated in regions further south (ODEPA, 2013; INE, 2014; ODEPA, 2020).

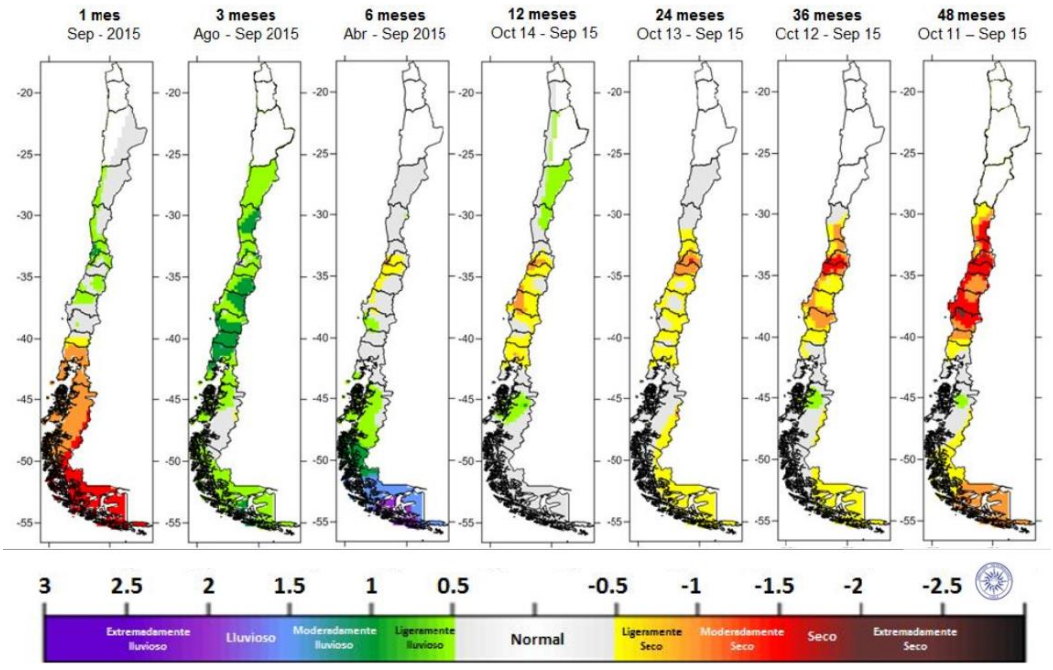

Figure 1. Projected drought in Chile, according to rainfall in 2015. (Source: DMC, October 2015).

In Chile, as well as other regions of the world, a greater proportion of land surfaces are under continual threat of desertification (transition of land towards greater aridity) as a result of anthropogenic activities and climate change (Laity, 2009). Because it is necessary maintain the food supply to the several billion people living on our planet, the production of high-quality food must increase every year, accomplishment particularly challenging in the face of global environmental change. Then, these requirements realize the necessity to develop new farming strategies, considering areas that would address the projected climate change at the farm level impact, to maintain or improve current food production, promote efficient water use and develop cultivation technologies to maintain the food supply, especially in places where climate conditions are prone to desertic features in the nearest future.

Among extreme environments, arid zones (deserts) are the most extensive landscapes of the earth's surface, occupying almost a third of it. Of this total, 53% correspond to hot (> 18° C) and 47% to cold (<18° C) zones (Mares,1999; Pointing & Belnap, 2012). Despite conditions of arid zones can locally vary around the globe, they are characterized by large fluctuations of day-night temperature, high UV- radiation, low availability of water (high evaporation rates and/or low precipitations), and low presence of higher organisms, such as animal and plants, compared with template environments (Köberl et al., 2011).

In this sense, desert plants have developed strategies to cope with the uncertain moisture availability. Shrubs and cacti represent a drought-tolerant strategy, while herbs, including both annuals and geophytes, may represent a drought-evader strategy. Drought-

tolerant plants rely heavily on the survival of above-ground parts and their re-sprouting ability to respond to moisture inputs, whereas drought-evaders depend entirely on underground dormant propagules (Armesto et al., 1993; Basu et al., 2019). In many cases, the association between microorganisms and plants in extreme environments, such as deserts, determines that plants and microorganisms can thrive (Grover et al., 2011, Verma et al., 2017).

In Chile, the Atacama Desert is about 1000 km long, extending from 20°S to 30°S along the Pacific coast of South America but is only a few hundred kilometres wide. It sits between the Andes Mountains and the Ocean Pacific coast. The Atacama Desert contains regions that represent the most extreme hyperarid soils known, and there are few studies reporting microbial community composition or distribution (Drees et al., 2006; Connon et al., 2007; Santhanam et al., 2012). However, research had currently been focused in to describe the overall microbiome of the Atacama Desert (Bull et al., 2016; Contador et al., 2020). Even when it is the driest desert in the world, the phenomenon known as "Flowering Desert" corresponds to an explosive bloom of dormant plants produced due to the presence of water, either as precipitation or fog (Vidiella et al., 1999). After rainfall, productivity may be extremely high, supporting a rich but short-lived biotic assemblage. However, the diversity and role of microbial communities in this short period of flowering are still unknown. The Flowering Desert is a phenomenon that occurs every 20 or 50 years, depending mainly on the action of "El Niño", which triggers rainfall covering the coast and nearby valleys (Armesto et al., 1993). This phenomenon has ecological and economic implications for the Atacama Region, representing a notorious source of resources for trophic webs in desert (*e.g.*, herbivores, pollinators) and domestic livestock, which depends on this short period of high vegetal productivity (Gutiérrez, 2008). With the current information available about the relevance of microbiome for the

plants (Drees et al., 2006) and vegetation dynamics in the flowering desert (Cabrera et al., 2015), it is possible to assume that there are interactions with microorganisms that favor the establishment and development of plants in this harsh environment.

Recent investigations have found that genomes, metagenomes, and metatranscriptomes of extremophile bacteria contain diverse genetic traits associated with the growth promotion and increase of stress tolerance of plants (Goswami et al., 2016; Yadav, 2017). Thus, studies focused on using extremophiles plant growth-promoting rhizobacteria (PGPR) as an alternative to increase the plant tolerance to abiotic stress and mitigate the effect of climate change appear as an attractive and environmentally friendly alternative to be considered and investigated. Beneficial microorganisms in soil microbiomes can increase crop productivity and the efficiency in using natural resources, even under plant stressing conditions, possessing the potential to be used as efficient biofertilizers in hot arid and semi-arid regions, where water quality or chemical fertilizers are not optimally used for crops production (Yadav et al., 2020).

To date, most investigations addressing microorganisms in the Atacama Deserts have been centered in hyperarid transects (Drees et al., 2006). However, evidence on the occurrence of beneficial bacteria in the rhizosphere of desert plants is scarce. It is widely accepted that a portion of bacteria associated with the rhizosphere can act as PGPR, improving the growth and tolerance of plants. In general terms, the PGPR functions can be divided into three different ways: synthesizing certain bioactive compounds required by plants (*e.g.*, phytohormones), facilitating the uptake of nutrients from the soil by releasing of organic acids or enzymes (*e.g.*, phosphomonoesterases), and/or preventing the action of phytopathogens, acting as a survival strategy under harsh conditions in the environment (Lugtenberg & Kamilova, 2009; Enebe & Babalola, 2018).

In deserts, it is known that bacteria having PGPR traits are present (Jorquera et al., 2014; Goswami et al., 2016). In Flowering Desert, the abundance and the potential role in nitrogen cycling of the bacterial community during desert bloom have been reported (Orlando et al., 2010). This finds suggest that vegetational patches stabilize the soil and increase nitrogen and carbon content available to sustain the development and activity of the overall microbial community (Orlando et al., 2012). This kind of beneficial bacteria might be applied in agriculture to enhance crop production in arid agro-ecosystems (Köberl et al., 2011) or agroecosystems under adverse climate events attributed to climate change, such as prolonged drought events. Hence, more profound knowledge on diversity and abundance of microbial communities in arid environments, as well as the abiotic factors influencing their metabolisms, would improve obtention of effective PGPR, adapted to more stressful conditions (Lugtenberg & Kamilova, 2009; Jorquera et al., 2014; Ruzzi & Aroca, 2015).

In hot arid and semi-arid zones, agriculture is mainly limited by the availability of suitable irrigation water and groundwater are the main sources of irrigation (FAO, 1986). In more technologized agriculture systems, some countries such as USA, Israel, Egypt and Spain, produce crops under highly dependent systems on water supply, and this dependence increases if crop production is intended to enhance in time. These enormous amounts of water and the expected impact on climate conditions are the major disadvantages on agriculture in the desert (Acosta-Martínez et al., 2008). However, another way to contribute to mitigation is increasing crop water productivity and drought tolerance and genetic improvement and physiological regulation in plants (Ali & Talukder, 2008), and biological community in soils to select microorganisms confer plant-promoting traits against water scarcity.

The bacteria recruited by plants give rise to diverse PGPR communities with common capabilities for improving plant functionalities (Soussi et al., 2016). Despite the presence of similar bacterial community in hot arid zones, plants can shape and select specific root-associated bacterial communities that include bacteria capable of coping with the abiotic stress of these ecosystems (el Zahar et al., 2008).

One of the best ways to evaluate the potential of environmental bacteria in controlled conditions is using a model plant. The model plant used will be selected based on the knowledge of it, implying that the tests or assays using it, only respond to the variables under study. Considering that most of cultivated tomato varieties are sensitive to water, temperature, and soil conditions in early phenological stages, which negatively affects the final production, tomato has been considered as a well plant model for PGPR research (Shamshiri et al., 2018). Cultivated tomato belongs to the Solanaceae family and the genus *Solanum*. They are not only the most popular vegetable worldwide (4.5 Million ha in 2014) but is the most studied fleshy fruit because it is easy to grow as a model plant (Schwarz et al., 2014). In Chile, tomato is the third most highly consumed fresh vegetable crop after corn and lettuce (INE, 2014). In addition, the evolution of the surface of tomato for fresh consumption at the national level increased from 5,463 in 2011, to 15,112 ha in 2019 (ODEPA, 2020). This could be due to favorable price scenarios achieved in the previous seasons. The low availability of labor and water scarcity were not limiting factors in expanding this crop in 2012, although it may have affected some individual producers (Flaño, 2013). Tomato is considered nationally as the most important vegetable. It is mainly produced in O'Higgins, Valparaiso, and Maule regions (Fig. 2).

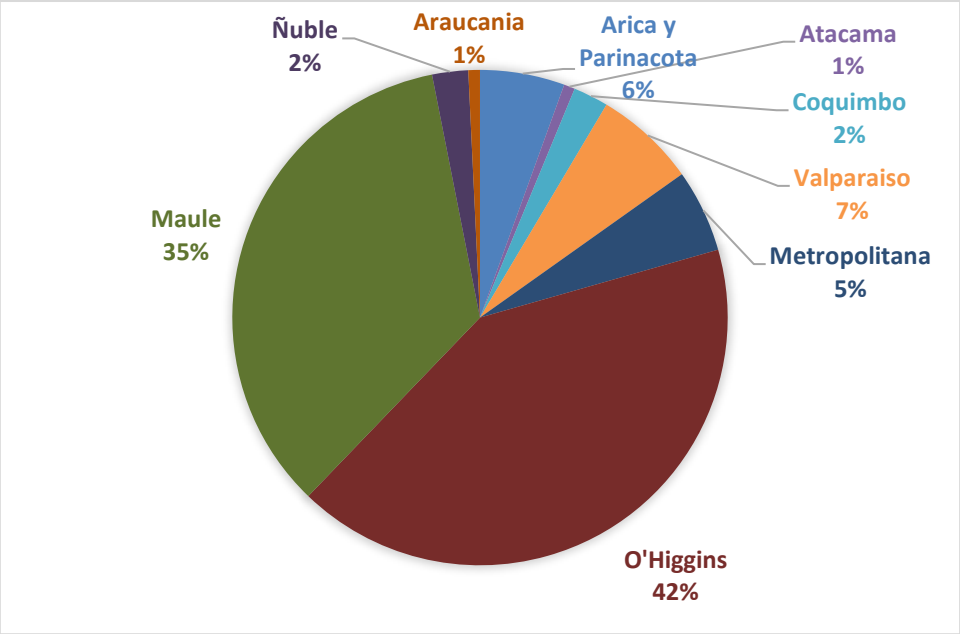

Figure 2. Area planted with tomatoes for fresh consumption in Chile. Source: ODEPA, 2019.

It is important to consider that the Valparaiso and O'Higgins regions are the main national tomato producers (Flaño, 2013). As shown in Figure 1, these regions face serious drought problems (Castro Avaria et al., 2014), which affect crop yields and producers' life quality (Roco et al., 2014). Therefore, the provision of biotechnological and applicable tools to maintain horticultural production in these regions becomes necessary.

The growth and flowering of tomatoes can be affected by biotic factors as well as by other environmental stresses, including high temperatures, water shortage, or soil salinity (Malash & Flowers, 2005). Thus, rapid growth and the early formation of flowers are a desirable for higher tomato production. In contrast, a delay in flowering can lead to delays and decreases in fruit production (Atherton & Rudich, 2013). Therefore, the development of microbial inoculants to improve the growth and flowering of tomatoes could represent valuable biotechnological tools for tomato cultivation throughout Chile. In this context, studies in India, Israel and other countries, have demonstrated that the

inoculation of rhizobacteria from dry salty environments can increase resistance in tomato to salt stress (Mayak et al., 2004a) and increase the weights (fresh and dry) of tomato seedlings exposed to transient water shortage (Mayak et al., 2004b). However, studies on the inoculation of rhizobacteria to mitigate the effect of water scarcity in Tomato cultivation in Chile have not been done thus far.

1.2 Hypotheses

Considering the projections on the impact of climate changes on Chilean agriculture and the contribution of rhizobacteria to ameliorate the adverse effect of water scarcity in plants, the following hypothesis is proposed:

"The application of potential plant growth-promotion rhizobacterial consortia from arid environments, such as flowering desert, will improve the growth of plants under stress by water scarcity."

1.3 General objective:

To describe the influence of culturable rhizobacteria associated with flowering desert, with plant growth-promoting capacities, on the growth of plants under stress by water scarcity.

1.4 Specific objectives:

1. To characterize the composition of total rhizobacterial communities associated with flowering desert phenomenon.
2. To formulate a rhizobacterial consortia with plant growth-promoting potential from culturable rhizobacteria associated with flowering desert phenomenon.
3. To evaluate the potential use of rhizobacterial consortia associated with flowering desert phenomenon to mitigate water scarcity stress in plants.

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CHAPTER II

Composition, predicted functions and co-occurrence networks of rhizobacterial communities impacting flowering desert events in the Atacama Desert, Chile.

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Composition, predicted functions and co-occurrence networks of rhizobacterial communities impacting flowering desert events in the Atacama Desert, Chile

1. Abstract

Flowering desert (FD) events consist of the rapid flowering of a wide variety of native plants in the Atacama Desert of Chile, which is categorized as the driest desert in the world. While ephemeral plants are an integral part of the desert ecosystem, there is little knowledge on plant-microbe interactions that occur during FD events. Consequently, the overall goals of this present study were to investigate changes in the composition and potential functions of rhizobacterial community of *Cistanthe longiscapa* (Montiaceae) during the 2014 and 2015 FD events and determine the composition, potential functions, and co-occurrence networks of rhizobacterial community associated with the root zone of *C. longiscapa* during pre- (PF) and full-flowering (FF) phenological stages. Results of this study showed that the Proteobacteria and Actinobacteria were the dominant taxa in rhizosphere soils during the three FD events (2014, 2015, and 2017) examined. In general, greater microbial richness and diversity were observed in rhizosphere soils during the 2015-, compared with the 2014-FD event. Similarly, predicted functional analyses indicated that a larger number of sequences were assigned to information processing (e.g., ion channel, transporters and ribosome) and metabolism (e.g., lipids, nitrogen, and sulfur) during 2015 compared with 2014. Despite the lack of significant differences in diversity among PF and FF stages, the combined analysis of rhizobacterial community data, along with data concerning rhizosphere soil properties, evidenced differences among both

phenological stages and suggested that sodium is a relevant abiotic factor shaping the rhizosphere. In general, no significant differences in predicted functions (most of them assigned to chemoheterotrophy, magnesium metabolisms, and fermentation) were observed among PF and FF. Co-occurrence analysis revealed the complex rhizobacterial interactions that occur in *C. longiscapa* during FD, highlighting to *Kouleothrixaceae* family as keystone taxa. Taken together this study shows that the composition and function of rhizobacteria vary among and during FD events, where some bacterial groups and their activity may influence the growth and flowering of native plants, and therefore, the ecology and trophic webs in Atacama Desert.

2. Introduction

The Atacama Desert, located in northern Chile (from 18°24'S to 29°55'S), is considered as the driest non–polar place on Earth (Clarke, 2006). The Atacama Desert is characterized by a combination of subtropical climate of high pressure and a cold coastal Humbolt current that creates a constant temperature inversion, offshore winds, and shadow effect that restrict moisture advection from east (Pacific Ocean) to west (Los Andes mountains) (Houston & Hartley, 2003; Clarke, 2006). However, the Atacama Desert is periodically filled with life and color in a phenomenon called the 'flowering desert (FD)', which corresponds to an explosive bloom of dormant desert plants produced by the presence of water as precipitation (Vidiella et al., 1999). During FD events (Fig. 1), also named as "blooming deserts" (Chávez et al., 2019), productivity may be extremely high, supporting a rich but short–lived biotic assemblage. The flowering occurs preferentially on mantles (Fig. 1C) of light wind–sands and, to a lesser extent, on the stony substrates of the plains that frame the sedimentary marine terraces, located between

100 and 300 meters above sea level. The FD events have relevant ecological, social, and economic implications for the Atacama region, activating trophic networks in the desert (e.g., herbivores and pollinators), which are resources for tourist activities and domestic livestock. The latter depends on this short period of high vegetal productivity (Gutierrez, 2008). Despite the ecological importance of FD, the impact of microbial communities in growth and flowering of desert native plants remain unknown, particularly those related to plants and their roots.

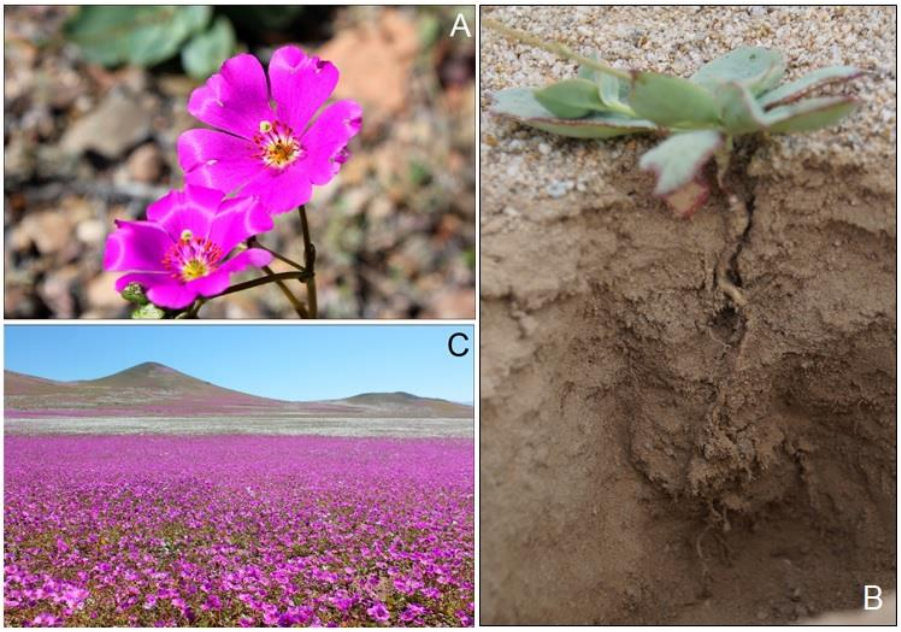

Figure 1. Flowers of *Cistanthe longiscapa* (A) and root in soil (B). Mantles of *C. longiscapa* during full-flowering (C) stage at 2017 flowering desert event in Atacama Desert.

While several studies have examined deserts around the globe for multiple inquiries (Beaulac et al., 2009), studies on flowering desert events are still scarce because of the issues about their occurring prediction, determined in most of the cases of the water available. In some deserts, the bloom of plants is an annually event (Cowling et al., 1999), while it is sporadically in others (Singh & Singh, 2011). Because of that, the main

rhizobacteria associated to these ephemeral plants, and their role in the ecology of this events have been scarcely reported. However, some studies in Sonoran Desert and Saudi Arabia have reported the important role of the plant biotic interactions in shaping the species diversity (Yasir et al., 2015; Franklin et al., 2016). In Atacama Desert, *Cistanthe longiscapa* (Barneoud) Carolin ex Hershkovitz is a member of *Montiaceae* family, is an endemic annual herb, and it is one of the most representative groups of native plants during FD events (Stoll et al., 2017). Nonetheless, information concerning its physiology and association with microorganisms are extremely scarce. Orlando et al. (2010), proposed that the bacterial community during FD bloom was likely involved in nitrogen (N) cycling. Moreover, plants and microbial communities have been shown to increase stabilization and contents of nutrients (N and carbon [C]) in desert soils (Orlando et al., 2012).

Recent high–throughput sequencing (HTS) studies have also described a wide diversity of bacteria associated with the rhizosphere (soil influenced by roots) of native plants in the Atacama Desert, including potential plant growth–promoting bacteria (Jorquera et al., 2014). However, there is a dearth of information concerning the diversity and role of rhizobacterial communities on the growth, survival, and fitness of FD plants, such as *C. longiscapa*. In this context, this study was designed in two stages: Firstly, we investigated the differences in composition and potential functions between rhizobacterial community of *C. longiscapa* during full–flowering (FF) stage in two consecutives FD events (2014 and 2015), and secondly, we investigated the composition, potential functions and co-occurrence networks of the rhizobacterial community associated with of *C. longiscapa* during the pre–flowering (PF) and FF phenological stages in a third FD event occurred on 2017, in order to elucidate the connections and key taxa in bacterial community during an unique FD event under two phenological stages.

3. Methodology

3.1. Stage 1: Composition and predicted functions of the rhizobacterial community during the 2014 and 2015 flowering desert events

3.1.1 Sampling: For this first stage of the investigation, rhizosphere soil samples were collected from three *C. longiscapa* mantles (about 50 to 70 m^2 each), located in three different locations (27º28'03''S, 70º50'22''W; 28º22'07''S,70º49'07''W; 28º46'10''S, 70º57'53''W) during the FF stages of local blooms in 2014 (October) and an extensive bloom in 2015 (September) FD events. The FF stage was defined as the period when > 90% of the plant mantles were in full bloomed. In each year, five rhizosphere soil samples (~20g, from 6 to 10 plants) were randomly taken in a 20 m transect of each mantle to a depth of 5 to 10 cm excavating the soil with a sterile trowel and removing live superficial roots (1−2 mm in diameter), including the soil that adhered to the roots (Fig. 1B). Rhizosphere soil was defined as that which adhered to the roots following shaking. The rhizosphere soil samples were deposited in sterile plastic bags, and immediately transported on ice to the Applied Microbial Ecology laboratory at La Frontera University. The rhizosphere soil samples were stored at –4°C until used for analysis.

3.1.2 Total DNA extraction: For DNA extraction purposes, homogenized rhizosphere soil samples (0.5 to 1 g) were pre–treated by vortexing for 1 h in 2 mL of sodium phosphate buffer (0.1 M, pH 8) and centrifuged at 16,000×g for 10 min (He et al., 2005). The pellet was subjected to cell disruption by bead–beating for 30 s using a Powerlyzer® homogenizer (Mo–Bio Laboratories, CA, USA), and the solution was subjected to DNA purification using Power Soil® DNA Isolation Kit (Mo–Bio Laboratories), according to

manufacturer instructions. The quality and quantity of DNA extracts were measured using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Inc., MA, USA). The DNA purity was assessed by determination of the A280/A260 absorbance ratios and only DNA extracts with absorbance ratios of ≥1.8 were used for bacterial community analyses.

3.1.3 High–throughput DNA sequencing: Purified DNA extracts from rhizosphere soil samples were initially submitted to Macrogen, Inc. (Seoul, Korea) for 454–pyrosequencing (Roche) using 16S rRNA as a gene target. The selection of primer set and sequencing of 16S rRNA gene libraries were according to the Macrogen, Inc. protocol and recommended for Roche 454 GS-FLX System using Titanium Chemistry (454 Life Sciences). Briefly, 16S rRNA gene libraries were prepared by PCR using the universal bacterial primer UNI_AMP–27F (5′–Zxxx GAG TTT GAT CMT GGC TCA G–3′ and UNI_AMP–518R (5′–K WTT ACC GCG GCT GCT GG–3′) (Lee et al., 2010), where Z and K represent two pyrosequencing primers (CCATCT CAT CCC TGC GTG TCT CCG ACT CAG and CCTATC CCC TGTG TGC CTT GGC AGT CTC AG), and xxx was designed for the sample identification barcoding key. The PCR reaction was as follow: a hot start at 95 °C for 3 min, PCR amplification was carried out for 35 cycles at 94 °C for 15 s, 55 °C for 45 s, and 72 °C for 1 min. A final extension step was carried out at 72 °C for 8 min. The 16S rRNA gene libraries were sequenced by with Roche 454 GS–FLX System using Titanium Chemistry (Roche Diagnostics Corporation, Life Sciences, Branford, CT, USA).

3.1.4 Analysis of rhizobacterial composition: The analysis of rhizobacterial community composition was done as described by Jorquera et al. (2016). Briefly, 454–

pyrosequencing data was processed and analyzed using QIIME software, version 1.9.1 (Caporaso et al., 2010). Denoising, filtering low quality reads (phred score > 25), trimming of the barcode sequences and chimera check were carried out according to standard operating procedures for QIIME (http://qiime.org/tutorials/tutorial.html). Raw sequencing data were deposited in the Sequence Read Archive (SRA) of NCBI under accession number SRR6461105.

Taxonomic assignment (at 97%) was carried out using UCLUST algorithm (https://www.drive5.com/usearch/manual/uclust_algo.html) and Greengenes Database Release 13_5 (http://greengenes.secondgenome.com/) (DeSantis et al., 2006). The relative abundance of bacterial taxonomic groups was computed and visualized by using Geneious version 7 (https://www.geneious.com/). Then, the richness (observed operational taxonomic units, Sobs) and diversity (Shannon and Simpson indexes) of total bacterial communities was analysed by using QIIME. In addition, the similarities between rhizobacterial communities were analysed based on a distance matrix constructed using Bray–Curtis calculator and visualized as a non-metric multidimensional scaling (NMDS) plot using R software (https://www.rproject.org/).

3.1.5 Prediction of rhizobacterial community functions: In order to obtain a first approximation of the metabolic potential of bacterial communities, a predictive functional analysis of rhizobacterial communities was performed by using PICRUSt software, version 1.1.0 (Langille et al., 2013). This analysis allows inference of the functional profile of a microbial community based on marker gene survey (16S rRNA gene), and we put attention in the differences among 2014 and 2015 FD events in some predicted functions with genetic (e.g., ribosomal content and tRNA biosynthesis), environmental information processing (e.g., signaling molecules and membrane transport) and

metabolism (e.g., C and N metabolism) relevance. Metabolic profile inference was performed to predict KEGG Orthology (KO) and Clusters of Orthologs Groups (COG) classes via the PICRUST software. The Nearest Sequenced Taxon Index (NSTI) was used to express the expected uncertainty in the predictions. The resulting functional profiles were analysed with the Tukey-Kramer post–hoc test and visualized using STAMP (Parks et al., 2014).

3.2 Stage 2: Composition and predicted functions of rhizobacterial community during 2017 flowering desert event

Considering the differences found in the composition and predicted functions of rhizobacterial communities among 2014 and 2015 FD events, a new sampling was done during the 2017 FD event, and focused in two different phenological stages (PF and FF) and adding interactive bacterial networks analysis. The PF stage was defined as the vegetative stage before the emergence of the floral buds, whereas FF stage was defined as > 90% of the plant mantles were full bloomed.

3.2.1 Sampling: Rhizosphere soil samples were collected during PF (August) and FF (September) stages in 2017 FD event. In this sampling, PF was defined as the stage before the appearance of floral buds and plants in vegetative growth. Rhizosphere soil samples (~20g) were collected and processed as described above and taken from the same locations of *C. longiscapa* during 2014 and 2015 FD events. Rhizosphere soil samples (~20 g) were subjected to chemical analysis as follow: soil pH was measured in 1:2.5 soil/deionized water suspensions. Available phosphorus (P-Olsen) was extracted using 0.5 M Na-bicarbonate and analyzed using the molybdate blue method (Murphy & Riley,

1962). Organic matter was estimated by the Walkley-Black method (Combs & Nathan, 1998). Exchangeable cations of potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were extracted with 1M ammonium acetate, pH 7.0, and analyzed by flame atomic absorption spectrophotometry (FAAS) (Warncke & Brown, 1998). The rhizosphere soil properties are shown in Table 1.

Table 1. Chemical properties of rhizosphere soils from *Cistanthe longiscapa* during 2017 Flowering Desert event.

Samples	**PF**[a]	**FF**
P_{Olsen} (mg kg^{-1})	21.2	20.9
Organic matter (%)	1.51	1.54
pH $_{H_2O}$	8.6	8.2
K ($cmol_{(+)}$ kg^{-1})	1.0	0.85
Na ($cmol_{(+)}$ kg^{-1})	0.61	0.62
Ca ($cmol_{(+)}$ kg^{-1})	75	81
Mg ($cmol_{(+)}$ kg^{-1})	2.0	1.85
CEC [b] ($cmol_{(+)}$ kg^{-1})	78.61	84.32

a PF: Pre-Flowering stage, FF: Full-Flowering stage.
b CEC=cation exchange capacity=Σ (K, Ca, Mg and Na).

3.2.2 Total DNA extraction and high–throughput DNA sequencing: Total DNA was extracted from rhizosphere soil samples by using Power Soil® DNA Isolation Kit (Mo–Bio Laboratories) according to manufacturer instructions as described above. Purified DNA extracts from rhizosphere soil samples were then submitted at Macrogen, Inc. (Seoul, Korea) for sequencing using 16S rRNA as gene target. It is necessary to mention that Macrogen, Inc. migrated from 454–pyrosequencing to Illumina technology for sequencing. The selection of primer set and sequencing of 16S rRNA gene libraries were

according to the Macrogen, Inc. protocol and recommended for Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA). Briefly, 16S rRNA gene libraries were prepared by PCR using the universal bacterial primers 27F-AGA GTT TGA TCM TGG CTC AG and 1492R-TAC GGY TAC CTT GTT ACG ACT T. The PCR reaction was as follow: a start at 96 °C for 1 min, PCR amplification was carried out for 25 cycles at 96 °C for 10 s, 50 °C for 55 s, and 60 °C for 4 min. A final step was carried out at 15 °C for until finished reaction.

3.2.3 Composition and predicted functions of rhizobacterial community: Sequence data was analyzed by using Mothur ver. 1.34.0 (Schloss et al., 2009). In brief, after trimming the low-quality regions at the ends of reads, reads were merged by using Fastq-join software (Aronesty, 2013), maintaining an average quality score >33. After removing the primer sequences, high quality sequencing reads were aligned on the basis of the Greengenes ver.13.8 database (McDonald et al., 2012). The UCHIME software was used to identify and remove probable chimeric sequences (Edgar et al., 2011). Raw sequencing data were deposited in the Sequence Read Archive (SRA) of NCBI under accession number from SRR9329822 to SRR9329839.

For statistical analysis, the Mothur program was also used to calculate alpha diversity indices, Shannon index and the abundance–based coverage estimate (ACE), in samples. A distance matrix constructed using Bray–Curtis calculator were used to analyze similarities between bacterial communities and visualized as a non-metric multidimensional scaling (NMDS) plot using the R software (https://www.rproject.org/). The VennDiagram package in R was used to identify shared OTUs of bacterial communities (Chen & Boutros, 2011) between different phenological stages of *C. longiscapa*.

Finally, to predict the potential function of microbial community among PF and FF stages, the FAPROTAX program was used to obtain the functional table through the default settings based on taxonomic information found in Antarctic vascular plant (Louca et al., 2016).

3.2.4 Co-occurrence network of rhizobacterial community during 2017 flowering desert event: The cooccurrence network was generated through the WGCNA package based on Spearman correlation matrix as described by Ma et al. (2016). The OTUs were represented by the nodes and the correlations between OTUs were described as the edges in the topological graph, respectively. During the network construction, the appropriate similarity (0.807) was chosen according to random matrix theory (RMT) (Luo et al., 2006). In addition, the Benjamini and Hochberg false discovery rate (FDR) was used to adjust the P values and set up the threshold value 0.05 (Benjamini et al., 2006). The topological network properties were calculated via igraph package (Csardi & Nepusz, 2006). Likewise, the keystone taxa were determined on the basis of thresholds: OTUs with degree higher 8, closeness centrality greater than 0.15, and betweenness centrality smaller than 0.08 (Berry & Widder, 2014). Gephi software was used to visualize the network image (Bastian et al., 2009).

4 Results

4.1 Composition and predicted functions of bacterial community during 2014 and 2015 flowering desert events

The relative abundances of the bacterial community in rhizosphere soil from samples obtained during 2014 and 2015 flowering desert events are shown in Fig. 2.

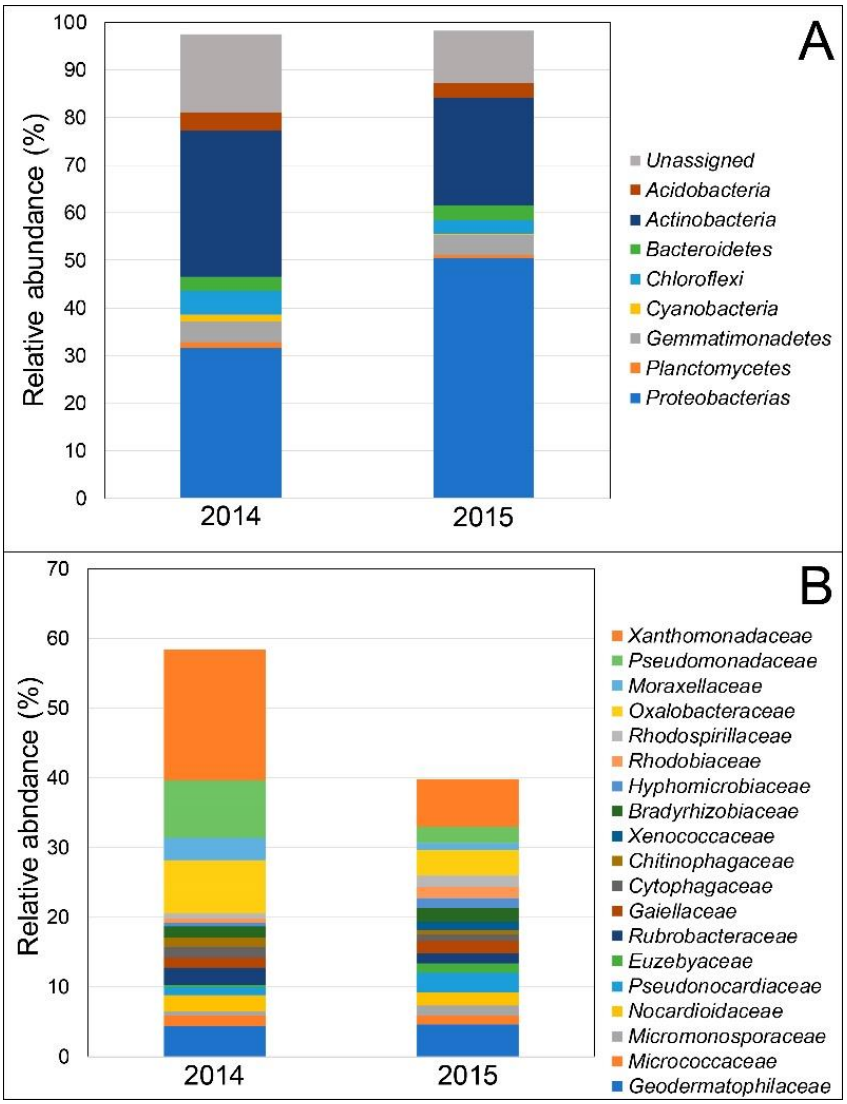

Figure 2. Average of relative abundance at phylum (**A**) and family (**B**) taxonomic levels in the total rhizobacterial community associated with *Cisthante longiscapa* during full-flowering stage of 2014 and 2015 flowering desert events in Atacama Desert.

At the phylum level (Fig. 2A), rhizosphere soil samples showed that Proteobacteria and Actinobacteria were the dominant phyla in 2014 and 2015, with values ranging from 31.6 to 51.0% and from 30.6 to 22.7%, respectively. The next abundant

phyla in rhizosphere samples were Chloroflexi and Gemmatimonadetes, with values ranging from 5.2 to 2.9% and from 4.3 to 4.2% during 2014 and 2015, respectively. Other phyla found among 2014 and 2015 samples were Bacteroidetes (2.9 to 3.0%), Acidobacteria (3.1 to 3.8%), Cyanobacteria (0,1% to 1.4%), and Planctomycetes (0.7% to 1.3%). At the family level (Fig. 2B), rhizosphere soil samples showed a higher abundance of Xanthomonadaceae (18.74%), followed by Pseudomonas (8.26%), Oxalobacteraceae (7.70%), Geodermatophilaceae (4.36%), and Rubrobacteraceae (2.50%) during 2014. Similarly, Xanthomonadaceae (6.78%) was the dominant family found in 2015, followed by the Geodermatophilaceae (4.68%), Oxalobacteraceae (3.68%), Pseudomonas (2.40%), and Rubrobacteraceae (1.55%). Bacterial community coverage ranged from 96 to 97% with a significant ($P \leq 0.05$) highest observed OTUs during 2015 respect to 2014 (Table 2).

Table 2. Coverage and alpha diversity among bacterial communities in the rhizosphere of *Cistanthe longiscapa* during full-flowering in 2014 and 2015 flowering desert events.

Year	Sample number	Coverage (%)	S_{obs} †	Shannon index	Simpson (D')
2014	5	97.25 ± 1.39 A* ‡	1665.00 ± 496.39 B	8.84 ± 1.36 B	0.9876 ± 0.012 B
2015	4	96.00± 1.25 A	2178.00 ±179.61 A	10.19 ± 0.2 A	0.9973 ± 0.001 A

†S_{obs}: number of OTUs observed at 97% similarity.
‡Values represent mean ± standard error.
*Different letter denotes statistical difference ($P \leq 0.05$, Tukey test) in the same column.

Significant higher ($P \leq 0.05$) values of diversity indexes (Shannon and Simpsons) were observed in 2015 compared with 2014. This difference between 2014 and 2015 FD

events was confirmed by NMDS, where rhizobacterial communities were more similar in 2014 than 2015 (Fig. 3).

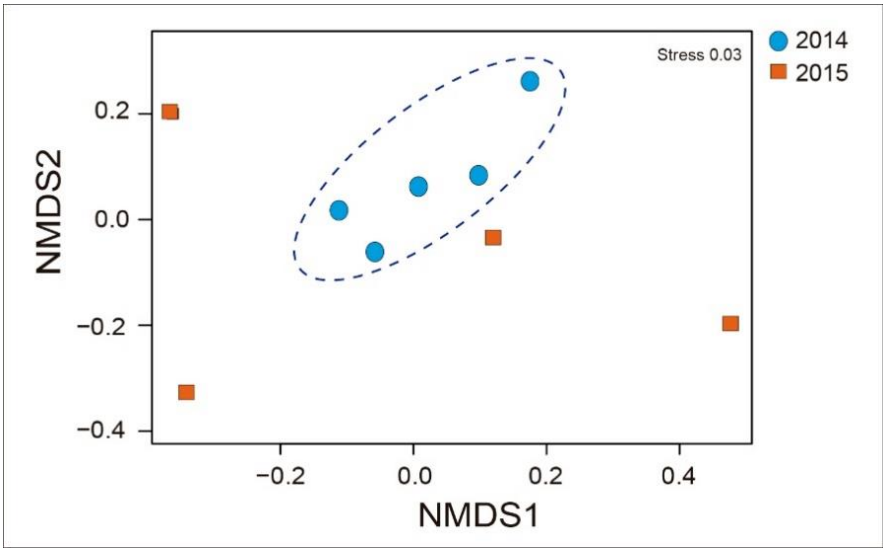

Figure 3. Nonmetric multidimensional scaling (NMDS) analyses derived from taxonomic data analysis of total rhizobacterial community associated with *Cisthante longiscapa* during full-flowering stage of 2014 and 2015 flowering desert events in Atacama Desert.

In addition, PICRUSt software allowed inference of differences in some genetic, environmental and metabolic functions among 2014 and 2015 events (Fig. 4). Thus, significant ($P \leq 0.05$) higher number of sequences were assigned to genetic information processing, such as ribosome protein expression and aminoacyl-tRNA biosynthesis, during 2015 in relation to 2014 (Fig. 4A and 4B). A significantly ($P \leq 0.05$) higher number of sequences were also assigned to environmental information processing, such as ion channels and transporters, during 2015 compared to 2014 (Fig. 4C and 4D). Similarly, a significantly ($P \leq 0.05$) higher number of sequences were assigned to energy metabolism (sulphur [S] and N metabolism) during 2015 in relation to 2014 (Fig. 4E and 4F).

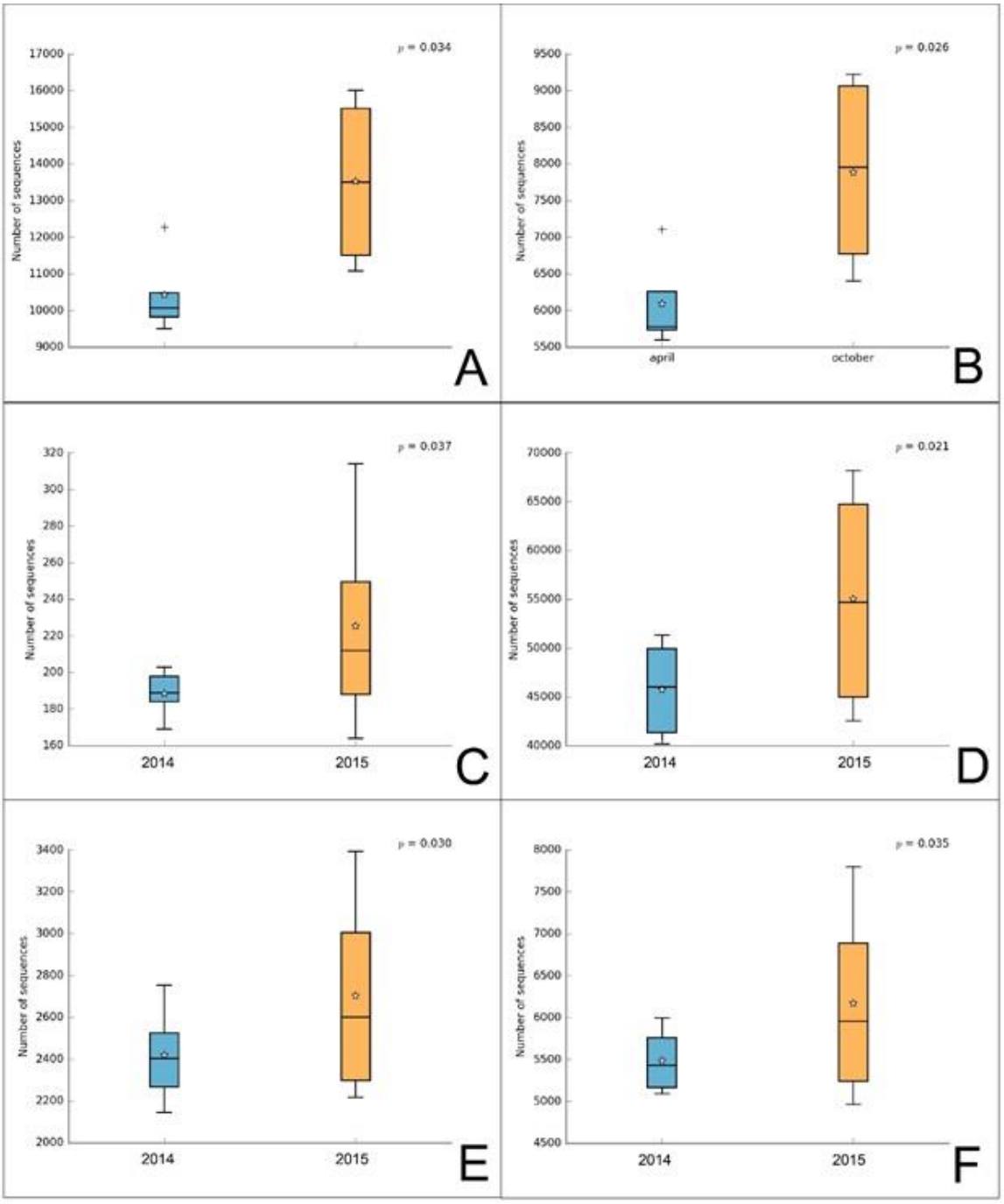

Figure 4. Number of sequences assigned to genetic information (ribosome [**A**] and aminoacyl-tRNA biosynthesis [**B**]), environmental (ion channels [**C**] and transporters [**D**]) and energy metabolisms (sulfur [**E**] and nitrogen metabolism [**F**]) in the total rhizobacterial community associated with *Cisthante longiscapa* during full-flowering stage of 2014 and 2015 flowering desert events in Atacama Desert.

4.2 Composition and predicted functions of bacterial community during 2017 flowering desert event

The relative abundances of total bacterial community in rhizosphere soil sampled during the PF and FF 2017 FD events are shown in (Fig. 5). As was found with 2014 and 2015 FD events, Actinobacteria and Proteobacteria were the predominant phyla in PF and FF rhizosphere soil during 2017 (Fig. 5A). However, significantly ($P \leq 0.05$) higher relative abundance of Actinobacteria was observed in FF (59.5%) than PF (49.6%) and yet significant smaller Proteobacteria were observed in FF (16.1%) compared with PF (19.5%). In terms of abundances, the Actinobacteria and Proteobacteria were followed by the Planctomycetes and Chloroflexi as dominant phyla, with values of 10 and 6 and 8 and 8% during PF and FF, respectively. Similar to previous FD events analyzed, other phyla found among the PF and FF were Bacteroidetes (4.2 to 3.5%), Acidobacteria (3.9 to 2.2%), and Gemmatimonadetes (1.6 to 1.3%%). Interestingly, greater abundances of these three phyla were observed in the PF than in FF, although there was not significantly different (ANOVA, $P \leq 0.05$).

With respect to minor taxa (< 1%), a higher abundance of bacterial groups was observed in PF compared with FF (Fig. 5B); mainly highlighting the Cyanobacteria (0.08 to 0.13%), WS2 (0.008 to 0.004%), Nitrospirae (0.21 to 0.15%) and FBP (0.21% to 0.12%) phyla in both phenological stages of plants, respectively. At the family level (Fig. 5C), a higher abundance of Bradyrhizobiaceae (2.8%) and Pseudonocardiaceae (2.1%) were found under PF compared with FF (2% and 1.7% respectively), contrasting with the relative abundance of Micrococcaceae (5.8%), Nocardioidaceae (3%) and Sporichthyaceae (1.8%) which showed higher abundance in FF (14%, 4% and 2.5%, respectively).

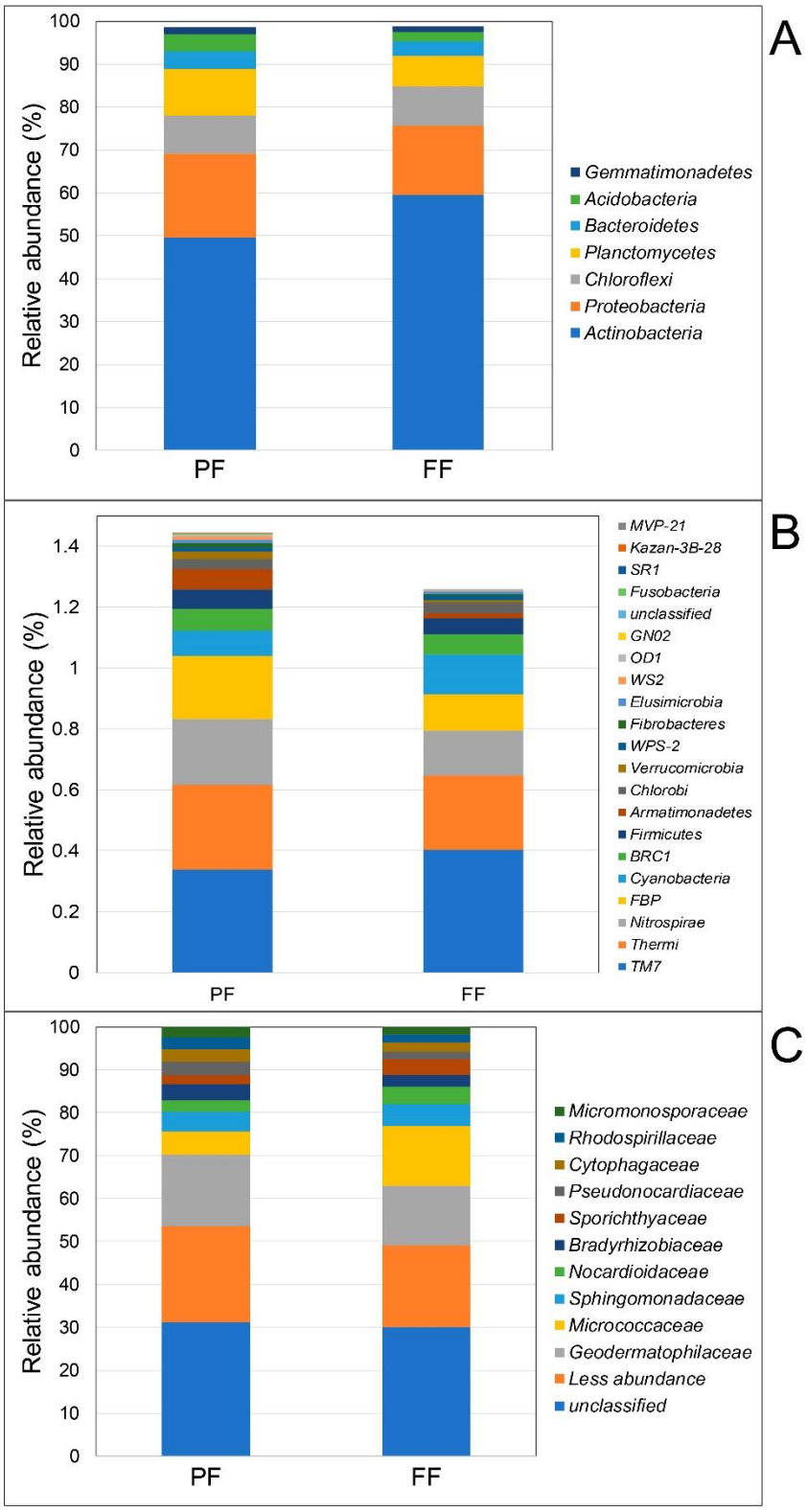

Figure 5. Average of relative abundance at phylum (major [**A**] and minor [**B**] abundance) and family (**C**) taxonomic levels in the total rhizobacterial community associated with *Cisthante longiscapa* during pre- (PF) and full-flowering (FF) stages of 2017 flowering desert event in Atacama Desert.

In relation to the bacterial communities, NMDS analysis showed two significantly different groups during PF compared with FF (ANOSIM, $P \leq 0.05$) (Fig. 6).

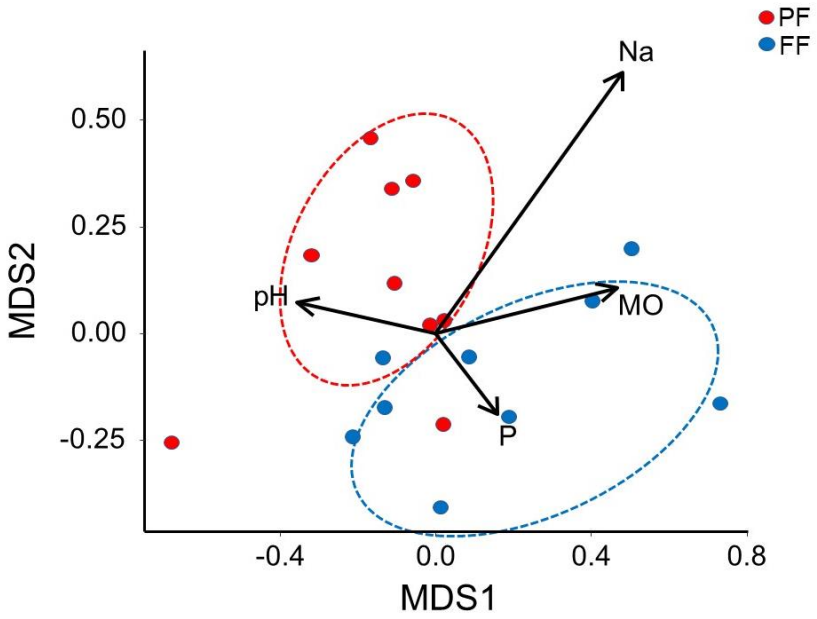

Figure 6. Non-metric multidimensional scaling (NMDS) analyses derived from taxonomic data of total rhizobacterial community and rhizosphere soil properties taken from *Cisthante longiscapa* during pre- (PF) and full-flowering (FF) stages of 2017 flowering desert event in Atacama Desert. O.M.: organic matter; P_{Olsen}: available phosphorus; Na: natrium.

Interestingly, Na content in rhizosphere soils was the key parameter regulating bacterial communities in both PF and PP (r^2 = 0.60, P = 0.01). Comparing the OTUs amount, only 28.0% (724 OTUs) and 23.3% (603 OTUs) were unique to PF and FF, respectively. About 2585 OTUs were shared among both stages. In addition, the percentage of coverage, the number of OTUs observed (97% similarity), and ACE were not significantly different between PF and FF stages (Table 3). In contrast, Shannon index was significantly greater (P = 0.05) in PF compared with FF.

Table 3. Coverage and alpha diversity among bacterial communities in the rhizosphere of *Cistanthe longiscapa* in 2017 flowering desert event at pre–flowering and full–flowering stages.

Stage	Sample number	Coverage (%)	Sobs[†]	Shannon index	ACE[‡]
Pre–flowering	9	98.99±0.07* [A**]	1533±79 [A]	5.85±0.1[A]	1851±102[A]
Full–flowering	9	99.16±0.07 [A]	1298±79 [A]	5.52±0.1[B]	1565±102[A]

[†] S_{obs}: number of OTUs observed at 97% similarity.
[‡] ACE: abundance-based coverage estimates.
[*] The values represent least squares mean ± standard deviation from n=9.
[**] Sample groups sharing the same letter in each column did not vary significantly ($P \leq 0.05$; Bonferroni test).

The FAPROTAX analysis predicted similar major functions among PF and FF, where major abundances were attributed to chemoheterotrophy (33.6 to 35.2%), aerobic chemoheterotrophy (32.3 to 32.8%), manganese oxidation (11.3to 10.3%), and fermentation (4.6 to 5.6%) (Fig. 7A). When minor predicted functions were taken into account, there were greater phototrophy (1.12% to 0.96%), nitrate reduction (1.16% to 1.23%), photoautotrophy (1.10% to 0.91%) and ureolysis (1.10% to 0.69%) in PF and FF (Fig. 7B). In a more detailed analysis, functions attributed to oxygenic photoautothrophy, N fixation and aromatic compounds degradation showed lower abundance in PF (0.16%, 0.16% and 0.47%, respectively) compared with FF (0.26%, 0.35% and 0.78%, respectively). It is particularly noteworthy that functions attributed to methane oxidation, dark hydrogen oxidation, aromatic hydrocarbon degradation, aliphatic non- methane hydrocarbon degradation, and aerobic anoxigenic phototrohpy presented the lowest abundances.

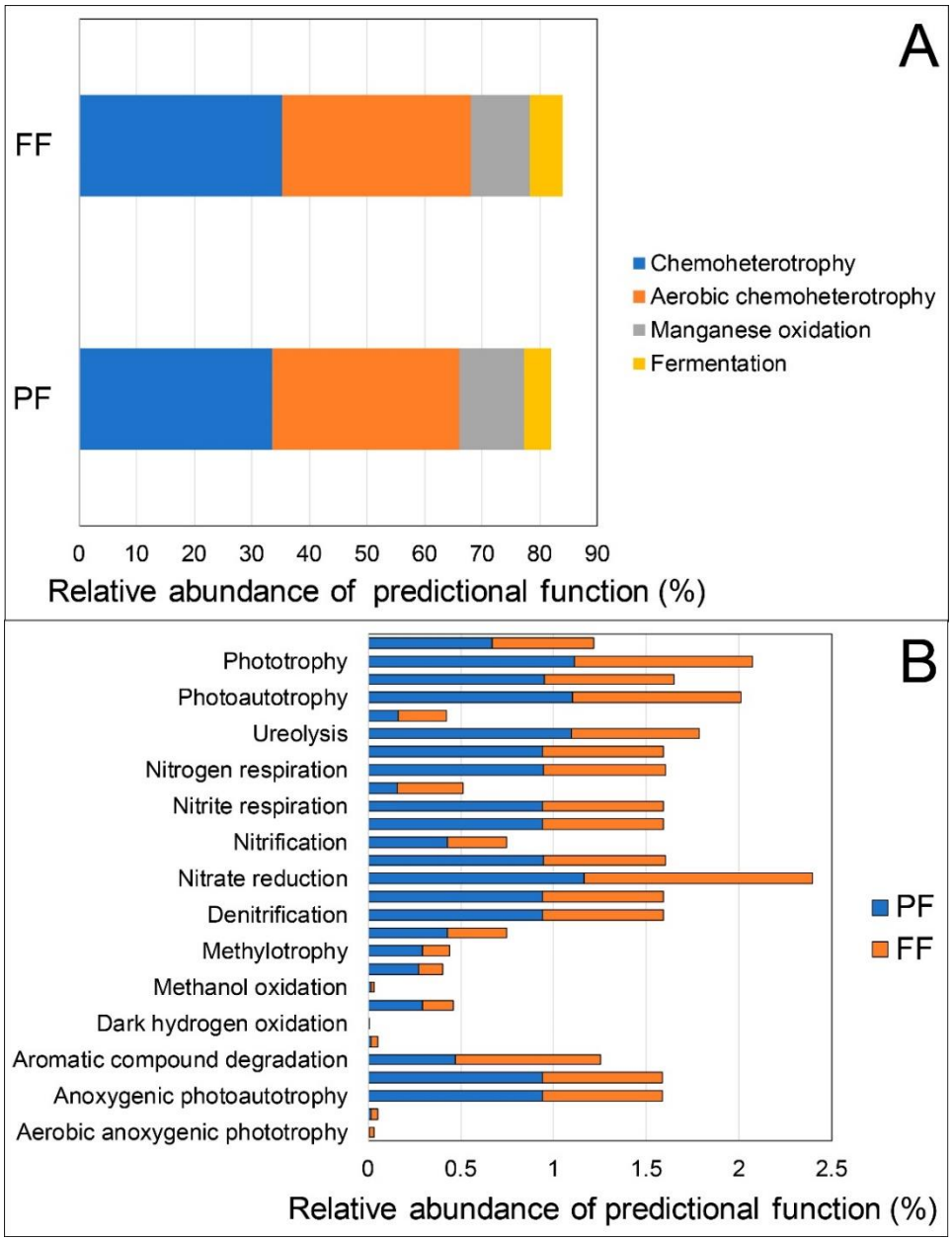

Figure 7. Average of relative abundance of major [**A**] and [**B**] minor functions predicted in the total rhizobacterial community associated with *Cisthante longiscapa* during pre- (PF) and full-flowering (FF) stages of 2017 flowering desert event in Atacama Desert.

4.3 Co-occurrence network of bacterial community during 2017 flowering desert event

The co-occurrence network of the rhizobacterial community of *C. longiscapa* included 970 nodes (e.g., OTUs) and 1324 edges, indicative of the association between OTUs (Table 4). The average network diameter, modularity index, and transitivity were 17, 0.809, and 0.19, respectively (Fig. 8). The results also show one keystone taxon, as the "engineering driver" leading the difference in the complex rhizosphere network between the two scenarios. This taxon (OTU) was classified into *Kouleothrixaceae* at the family level. Despite its importance, the abundance of this keystone taxon was only 0.02% in FF and was not detected in PF.

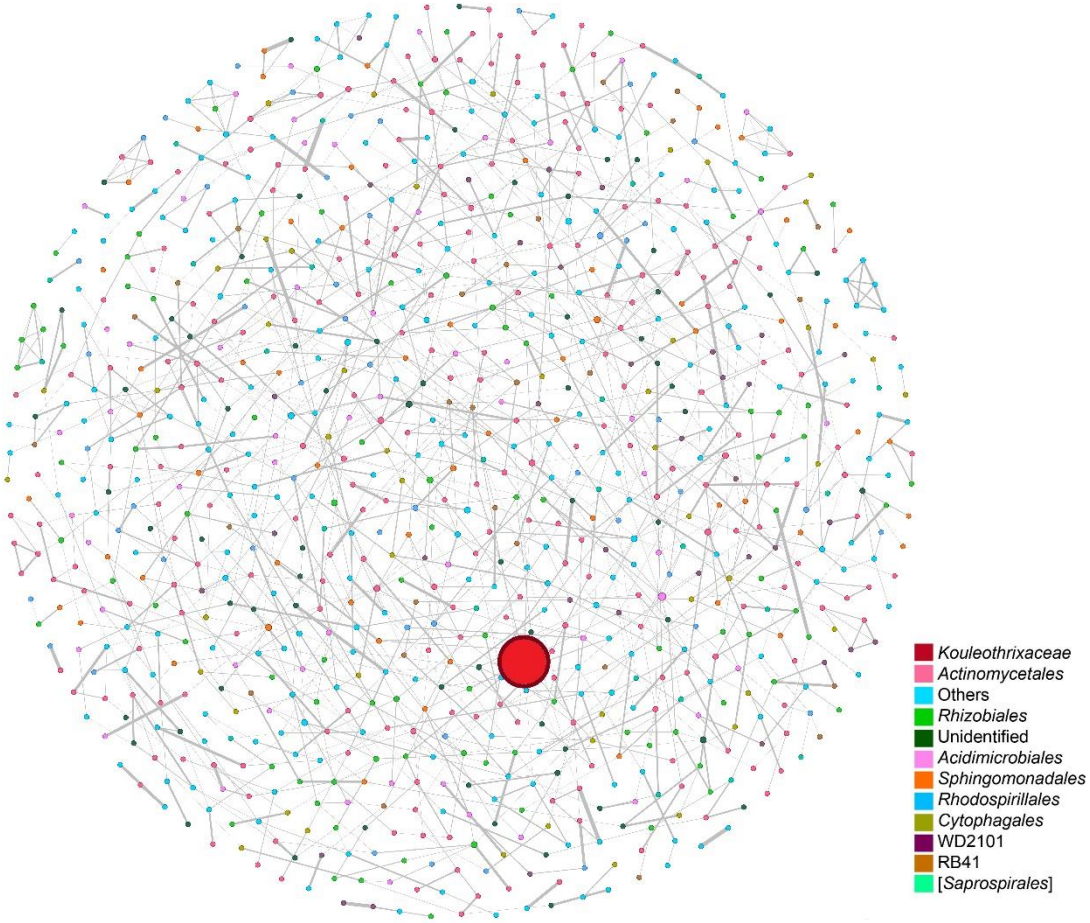

Figure 8. Co-occurrence network analysis of the total rhizobacterial community associated with *Cisthante longiscapa* during pre-(PF) and full-flowering (FF) stages of the 2017 flowering desert event at the Atacama Desert. The size of each node (representing OTUs) is proportional to the number of connections (degrees). The size of edges connecting nodes represent both strong (spearman's $\rho > 0.807$) and significant ($P \leq 0.05$) correlations between OTUs. Node colors represent the taxa indicated.

Table 4. Co-Occurrence Network properties of the rhizobacterial communities associated with *Cisthante longiscapa* during pre- and full-flowering stages of 2017 flowering desert event in Atacama Desert.

Parameter	Value
Vertex number	970
Edge number	1.324
Diameter	17.438
Average path length	8.873
Average nearest neighbor degree	3.154
Betweenness centralization	0.059
Closeness centralization	0.002
Density	0.002
Degree centralization	0.006
Degree assortativity	0.104
Transitivity	0.193

5. Discussion

Results of this current study showed that rhizobacterial community compositions associated with *C. longiscapa* during 2014 and 2015 FD events were determined by the presence of *Proteobacteria* and *Actinobacteria* as main phyla. Similarly, *Proteobacteria* and *Actinobacteria* have previously been reported as dominant phyla in the rhizosphere of native plants (*Atriplex* sp. and *Stipa* sp.) in Atacama Desert by using denaturing gradient gel electrophoresis (DGGE) and 454–pyrosequencing of 16S rRNA genes (Jorquera et al., 2014 and 2016). By using Illumina MiSeq sequencing of the 16S rRNA gene, rhizobacterial communities associated with Atacama Desert high altitude native plants (*Calamagrostis crispa*, *Nassella nardoides*, *Jarava frigida* and *Pycnophyllum*

bryoides) were dominated by *Proteobacteria*, whereas *Actinobacteria* was dominant in bulk soils (Fernández-Gómez, 2019). In FD events during 2005 and 2006, Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes were found as dominant phyla by using cloning and sequencing of the 16S rRNA and ammonia monooxygenase (*amo*A) genes (Orlando et al., 2010).

Here we show that the Proteobacteria and Actinobacteria were followed by Chloroflexi and Gemmatimonadetes as dominant phyla. Despite the differences in relative abundances, Chloroflexi seems to be present in most of the soils around the including the rhizosphere microbiomes (Mendes et al., 2011). Members of Chloroflexi has also been found in desert soil, including various soil communities and hypoliths of the Atacama Desert (Neilson et al., 2012). With respect to Gemmatimonadetes, it is known that this bacterial group can thrive in hyperarid soils with extremely low organic C and N levels, suggesting that their abundance in arid soils implies that they are important colonists (DeBruyn et al., 2011). Gemmatimonadetes have also been described as common inhabitants of rhizosphere soils in Chilean ecosystems (Jorquera et al., 2010), including FD event (Orlando et al., 2010). Other phyla found as component of *C. longiscapa* rhizosphere included Bacteroidetes, Acidobacteria, Cyanobacteria and Planctomycetes. These taxa have been found in arid and semiarid regions of the world, including cold deserts in the Antarctic (Aislabie et al., 2006), hot deserts in Chile (Navarro-González et al., 2003), and undisturbed and agro-ecosystems (Jorquera et al., 2014; Tian et al., 2015).

At family the level, *Xanthomonadaceae* was the principal bacterial family detected in the 2014 FD event, but with a lower abundance than in the 2015 FD event. *Xanthomonadaceae* family is recognized as typical soil bacteria (Assis et al., 2017) and members of this taxa can be found in several environments, including rhizosphere soils

(Pétriacq et al., 2017). Other families, such as *Oxalobacteraceae*, *Geodermatophilaceae* and *Rubrobacteraceae*, were also found in both FD events, but in different proportions. Moreover, *Pseudomonas*, *Oxalobacteraceae* and *Rubrobacteraceae* diminished in 2015 compared to 2014, and *Geodermatophilaceae* was found in the same proportion for both FD events. The family *Geodermatophilaceae* harbors many strains adapted to extreme ecological niches, such as the desert core in the Atacama Desert (Castro et al., 2018), and at high altitudes and in hyper-arid soils of the Chilean Central-Andes (Bull et al., 2018). Coincidently with 2014 and 2015 FD events, 2017 FD event showed Proteobacteria and Actinobacteria, followed by Chloroflexi and Planctomycetes as dominant phyla in both PF and FF stages.

In relation to rhizobacterial diversity, alpha diversity measured by the Shannon index (H′) showed significant differences among 2014 and 2015 FD events, with values of 8 and 10, respectively. Similar values were obtained in the rhizosphere of invasive plants by Cheng et al. (2019), which rapidly grow and are in constant expansion. The Simpson index, as well as H', corroborated the differences in rhizobacterial diversity for both FD events, with values lower (0.98 and 0.99) to those found in rhizosphere of desert plants (2.3 to 3.9) (Aguirre-Garrido et al., 2012) and comparable to those obtained for plants with agricultural importance (0.87 to 0.97) (Yang et al., 2016).

Differences in rhizobacterial communities among 2015 and 2014 FD events were also confirmed by NMDS analysis derived from taxonomic data. A higher-level grouping in the rhizobacterial communities was evidenced during 2014 compared with 2015 FD, which suggests that rhizobacteria bacterial communities had similar composition and maybe exert similar functions in rhizosphere during flowering of *C. longiscapa* Shifts in functions of bacterial community might not be coincident with changes in taxonomic as observed by Jorquera et al. (2014) and Marileo et al. (2016) using phylogenetic (16S

rRNA and *rpo*B) and functional (*amo*A, *nif*H and APase) genes. In this context, rhizobacterial community functions predicted by PICRUSt shows significant differences ($P \leq 0.05$) in numbers of sequences involved in environmental and genetic information processing among 2014 and 2015 FD events. A greater number of sequences were assigned to ion channels, transporters, ribosome and aminoacyl–tRNA biosynthesis in 2015, compared with 2014. Ion channels and transporters are formed by proteins and are responsible to regulate ion transportation (Na^+, K^+, Ca^{2+} and Cl^-) and molecules (ethanol, urea, amino acids, glucose 6–phosphate, among others) through cellular membranes maintaining electrochemical gradient and volume of bacterial cells (Potts, 1994). Transport of ions is crucial to prevent cellular damage caused by dehydration or desiccation, especially in hot and desert environments, where the amount of salts and ions in soils, compared with intracellular composition in bacteria, is higher favouring the loss of water in bacterial cell (Rothschild & Mancinelli, 2001; Zhang & Yan, 2012).

Differences in energy and metabolism of S and N during 2014 and 2015 FD events were also predicted. Higher number of assigned sequences related to the abovementioned functions were observed during 2015, compared with 2014 FD event. Similarly, to ribosomes and aminoacyl-tRNA biosynthesis, S and N metabolisms are involved in proteins synthesis. N metabolism is also responsible of essential processes in the rhizosphere such as N fixation, nitrate reduction, denitrification, nitrification, etc. (Klemme, 1989; Richardson & Watmough, 1999). In addition, microbial colonization is the predominant form of primary productivity and N input in such extreme desert regions (Lacap et al., 2011). Interestingly, even though two different platforms for high-throughput sequencing were used for rhizobacterial community analyses in this research, both provide a comparable view of the community sampled, regardless of differences in read length and sequencing technologies, as demonstrated by Luo et al. (2012). Regarding

PF and FF, some significant shifts in the *Proteobacteria* and *Actinobacteria* abundance were observed. Actinobacteria increased in their relative abundance in FF while Proteobacteria decreased. Chloroflexi had a similar relative abundance in both stages and Planctomycetes was less abundant in FF than PF. These changes in the composition of total rhizobacterial communities during PF and FF may be explained by the metabolic differences according to plant phenological stages (Houlden et al., 2008). Some investigations observed changes not only in the structure but also in the functionality of culturable rhizobacterial communities during different phenological stages of plants (Ruiz-Palomino et al., 2005). In *C. longiscapa* rhizosphere, assignations of *amo*A gene to different *Nitrospira* clusters were observed before, during and after FD events (Orlando et al., 2010). In addition, it is commonly reported that an increase in the secretion of proteins, organic acids, and phenolic compounds by plant roots during the flowering stage (Lucas García et al., 2001), inducing a higher growth and activity of bacteria as well as the recruitment of specific bacterial groups in the rhizosphere (Lundberg et al., 2012; Edwards et al., 2015).

At the family level, the dominant groups were *Bradyrhizobiaceae, Pseudonocardiaceae, Micrococcaceae*, *Nocardioidaceae, Sporichthyaceae*, at different proportions in both PF and FF stages during 2017 FD event. In the case of *Bradyrhizobiaceae and Pseudonocardiaceae,* both families were present in less abundance in PF than in PF. *Bradyrhizobiaceae* is known for the N fixation capacities of their representatives as one of the most important ecological properties with potential application in agriculture besides other diazotrophic members into the *Alphaproteobacteria* class (Marcondes de Souza et al., 2014). In relation to *Pseudonocardiaceae*, some of their representatives are able to produce bioactive compounds with antimicrobial activity, and to thrive under strongly UV-B

irradiation (Bull et al., 2018). Thus, the aforementioned rhizobacterial families could be important collaborators for the establishment of a plant cover in the desert soil and their shift in abundance may be explained precisely for these features. In contrast, families *Micrococcaceae, Nocardioidacea* and *Sporichthyaceae* increased in abundance in PF. These abundance changes could also be associated with the principal features of each family where *Micrococcaceae* and *Nocardioidaceae* harbor representatives considered the most abundant degraders of plant residue and primary degraders of organic material in crop lands (Ortiz-Cornejo et al., 2017) and have a significant role in degradation processes and nutrient cycling (Tóth & Borsodi, 2014). This degrading capacity would allow to these rhizobacterial groups to be more abundant in FP than FF. Interestingly, the *Sporichthyaceae* have been described as solar radiation tolerant microbes and found in impoverished soils (Bull et al., 2018). However, *Sporichthyaceae* is one of the less studied bacterial group mostly owing to their slow growth rate and requirements (Normand, 2006).

In relation to the diversity of rhizobacterial community among the FF and PF, no differences were observed in both phenological stages, except with Shannon index. However, differences among phenological stages were evidenced when rhizobacterial communities were evaluated with some rhizosphere soils properties using NMDS analysis. The grouping pattern by NMDS is consistent with the greater supply of nutrients for bacteria during FF (secreted by the plant, and the recycling of plant detritus), which modulates the variety of bacteria inhabiting this niche (Miki et al., 2010). In contrast, during PF, when fewer resources are available, a higher competence between bacterial groups would occur, hence the relative abundances at phyla level could vary drastically among phenological stages. NMDS also showed sodium (Na) as the main abiotic factor influencing the rhizobacteria grouping, more than organic matter (O.M.), available

phosphorus (P_{Olsen}) or pH. Atacama Desert soils are characterized by their high salinity where commonly are isolated salt tolerance culturable bacteria (4% to 8% NaCl) (Okoro et al., 2009; Maza et al., 2019). Factors influencing the rapid variation and adaptation of rhizobacterial communities are identified as plant genotype, phenological stages of plants, composition of rhizodeposits, bacterial assemblages, microenvironment generated by a whole set of plants or bacterial interactions (Fernandez-Gomez et al., 2019), especially considering that some bacterial taxa observed in this study are also influenced in their distribution and diversity in short periods of time by diurnal cycling (Staley et al., 2017).

Analysis of predicted functions in rhizobacterial communities showed that the greatest amount of sequences was assigned to chemoheterotrophy, aerobic chemoheterotrophy, manganese oxidation and fermentation in both phenological stages. These metabolic processes are related to energy production from different metabolic sources, explained by C-containing primary and secondary metabolites from the root exudates (Bais et al., 2006), low- and high-molecular weight compounds, proteins, organic acids, sugars and some polysaccharides (mucilage), which must be metabolized by rhizobacteria in order to obtain energy for microbial survival (Walker et al., 2003; Ladwig et al., 2015). In addition, minor functional predictions were mainly assigned to different metabolic processes, such as photoautothropy and N metabolism (*e.g.*, nitrate reduction and ureolysis), processes related to N fixation and N transformation in soil (Oshiki et al., 2018).

The co-occurrence network of the rhizobacterial community of *C. longiscapa* revealed the presence of 970 nodes and 1324 edges. These results were higher to other rhizosphere analyses in desert plants, were nodes range from 375 to 488 (Gunnigle et al., 2017). In general, these values are similar to those found in rhizosphere of some Mediterranean plants with values ranging from 350 to1000 nodes (Shi et al., 2016). In the

same study, the amount of edges for *Avena fatua* was lower (40 to 1200 edges) than the amount obtained for *C. longiscapa*, during the 2017 FD event, indicating more connections between different bacterial taxa. In addition, the modularity index was 0.809, similar to those obtained for plants growing under desert environments affected for monsoon climate (Zheng et al., 2017) indicating that the network has a modular structure (Newmann, 2006), with groups of highly connected nodes within the group and few connections outside the group (Barberán et al., 2012). The networks also showed to members of *Kouleothrixaceae* family (*Chloroflexi* phylum) as keystone taxon in the *C. longiscapa* rhizosphere, despite that its abundance was only 0.02% in FF and did not detected in PF. In other environments (such as sludge), *Kouleothrixaceae* representatives have been characterized as bacteria specialized in polysaccharide degradation produced by other microorganisms and on decaying cells (Kragelund et al., 2007), which is an important feature for bacteria in arid environments, particularly in rhizosphere, where C sources are limited to plant exudates, EPS produced for microorganisms and plant debris. Members of the family *Kouleothrixaceae* have been reported in the rhizosphere of Nickel (Ni)-hyperaccumulator plants, but there is no correlation between the relative abundance of *Kouleothrixaceae* and metals (Niquel), cations (Cadmium) and O.M. was found (Lopez et al., 2017).

Despite the low abundance of members of the *Kouleothrixaceae* family, they are likely still important for ecosystem function. Several studies currently highlight the relevance of low abundance and rare taxa in nature, indicating population dynamics, dispersion, predation and persistence of these underrepresented bacteria (Lynch & Neufel, 2015). Moreover, some studies exploring the microbial diversity from Atacama Desert soil, have reported that rare taxa are able to contribute in the dynamic (Shade et

al., 2014) and resilience (Idris et al., 2017) of the total soil bacterial community acting as a reservoir that can rapidly respond to environmental changes.

6. Conclusions

Flora and fauna living in Atacama Desert are highly adapted to local harsh conditions, where flowering desert (FD) events and their associated bacterial communities are pivotal for the ecology, tourism and domestic livestock production of the Atacama region. In this study, the analysis of the bacterial communities revealed that Proteobacteria and Actinobacteria phyla are the dominant taxa in the *C. longiscapa* rhizosphere among and during (FD) events. However, significant differences in the composition of total rhizobacterial communities were revealed not only among the 2014 and 2015 events but also among pre- (PF) and full flowering (FF) stages during 2017 FD event. Similarly, higher number of predicted functions (information processing and metabolism) were assigned to 2015 compared with 2014 FD event, but no big differences in predicted functions were found among PF and FF stages during 2017 FD event, where chemoheterotrophy, manganese oxidation and fermentation represented the major assignations. The co-occurrence network analysis also revealed the complex bacterial association in *C. longiscapa* rhizosphere during FD events, highlighting Kouleothrixaceae family as key stone taxa with higher number connections within community, but with a low abundance (0.02%). Our results not only reveal the compositions and potential functions of bacterial communities but also the relevance of minor taxa (or rare taxa) impacting rhizosphere processes for fast growth of native plants during FD, which is one of the most extraordinary (and scarcely studied) natural event in Atacama Desert, one of the driest places in the globe.

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CHAPTER III

Flowering Desert Event (Atacama Desert, Chile) rhizobacteria contribute to mitigation of water scarcity stress during germination and growth of tomato seeds.

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Flowering Desert Event (Atacama Desert, Chile) rhizobacteria contribute to mitigation of water scarcity stress during germination and growth of tomato seedlings.

1. Abstract

Tomato (*Solanum lycopersicum* L.) is an important vegetable cultivated around the world. Under field conditions, tomato can be negatively affected by water scarcity in arid and semiarid regions. Application of native plant growth–promoting rhizobacteria (PGPR) isolated from arid environments has been proposed as inoculants to mitigate abiotic stresses in plants. In this study, we evaluated rhizobacteria from *Cistanthe longiscapa* (*syn Calandrinia litoralis* and *Calandrinia longiscapa*), a representative native plant of flowering desert (FD) event (Atacama Desert, Chile), for their ability to reduce water scarcity stress on tomato seedlings. The isolated, bacterial strains were characterized with respect to their PGPR traits and included P-solubilization, 1-aminocyclopropane–1–carboxylate deaminase activity, and production of tryptophan-induced auxins and exopolysaccharides. Three PGPR consortia were formulated with isolated *Bacillus* strains and then applied to tomato seeds and then, seedlings were exposed to different levels of water limitations. In general, tomato seeds and seedlings inoculated with PGPR consortia resulted in significantly ($P \leq 0.05$) greater plant growth (48 to 60 cm of height and 171 to 214 g of weight) and recovery rates (88 to 100%) than those without inoculation (37 to 51 cm of height; 146 to 197 g of fresh weight; 54 to 92% of recovery) exposed at time intervals (24, 72 and 120 h) of no irrigation before transplantation. Our results reveal the effectivity of formulated PGPR consortia from FD to improve the performance of inoculated seeds and seedlings subjected to water scarcity, which can represent an

alternative to farmers, facing the drought events and water scarcity associated with climate change in agriculture of semiarid and arid regions worldwide.

2. Introduction

Currently, climate change is one the main concerns for agriculture worldwide. Extensive drought periods and eventual heat waves have been attributed to climate change, provoking significant losses in agriculture, especially in arid and semiarid regions in the globe (Morton & Anderson 2008; Davis, 2016). In Chile, the mayor risk is desertification processes, which directly affect the vegetable production of the country, because of the persistent drought affecting the country for the last 10 to 15 years (Quintana, 2000; Garreaud et al., 2020). To solve this limitation, studies have proposed the search, selection and use of cultivars with a higher tolerance to stress because of water scarcity; however, this alternative is time and cost consuming for many extensive agricultural areas in arid and semiarid regions affected by climate change (Kumar et al., 2008; Mancosu et al., 2015). In the same sense, the farmers have been forced to maintain or improve the food supply in areas establishing irrigation systems to prevent water shortage stress in orchards; however, the use of low-quality water (e.g., rivers, estuaries and underground) have increased the salinity of soils also affecting the growth and performance of plants (Pérez-Sirvent et al., 2003; Chartzoulakis & Bertaki 2015). Thus, efficient strategies to overtake the effect of climate adverse events, such as droughts with the consequent water scarcity and higher dehydration in plants, are highly required. Over the last decade, the relevance of microbiome-based science and plant growth–promoting rhizobacteria (PGPR) has been demonstrated by diverse studies in agricultural–relevant plants (such as crops, pastures, cereals, fruit trees, etc.) (Grover et al., 2011; Pérez-Montaño et al., 2014;

Basu et al., 2021). The occurrence of PGPR not only have been reported in agriculturally relevant plants, but also in native plants living in environments characterized by a permanent low availability of water and nutrients (Bashan et al., 2009; Jorquera et al., 2012, 2016, 2018). In these environments, the native plants and their microbiota have co-evolved under arid conditions; therefore, recent studies have proposed the use of PGPR from arid environments as inoculants to mitigate the damage of water limitation (drought) stress in plants. In this context, inoculation of wheat with consortia of PGPR isolated from the Atacama Desert (AD) improved the plant growth under water shortage conditions (Inostroza et al., 2017). While these results were encouraging, major efforts are still required to validate and implement this strategy at commercial scale used in agriculture in arid and semiarid regions.

In Chile, the Flowering Desert (FD) phenomenon, also known as blooming desert, an event triggered by short and infrequent rainfalls, mostly in southern border of the Atacama Desert, producing an increase of soil water available (Connon et al., 2007; Mukhtar et al., 2019; Chavez et al., 2019). This results in the explosive growth of native plants where the productivity may be extremely high and supporting a rich, but short-lived, biotic assemblage (Vidiella et al., 1999; Orlando et al., 2010). While a few studies have described the composition and functionality of rhizobacterial in plants during FD, most of ecological implications remain unknown. Moreover, even fewer studies have examined the AD as source of PGPR that promote the fast growth and prevalence of plants in changing environments, and their potential use for agriculture under water limiting conditions. Thus, considering that agricultural production in tempered and Mediterranean regions facing water scarcity problems impacted by climate change related events, and the potential use of PGPR from arid environments to enhance vegetable production in this areas, we isolated and formulated rhizobacterial consortia from

Cistanthe longiscapa (Barnéoud) Carolin ex M. A. Hershkovitz a representative, native, and wide spread plant during FD events, and then evaluated their effect on the seed germination, recovery and growth of tomatoes seedlings subjected to stress by water scarcity, as tomato is one of the most fresh consumed vegetables in Chile and its final production is very sensitive to environmental stresses at initial phenological stages (ODEPA, 2021).

3. Material and Methods

3.1 Sampling

Rhizosphere soil samples were collected from three locations (27º28’03’’S, 70º50’22’’W; 28º22’07’’S,70º49’07’’W; 28º46’10’’S, 70º57’53’’W) during a FD event that occurred in the southern border of the Atacama Desert, Chile in 2017. Rhizosphere soil samples from representative mantles of *C. longiscapa* (Barnéoud) Carolin ex M. A. Hershkovitz (*C. longiscapa*) (Fig. 1) were collected using a cleaned spade to a depth of 0–10 cm as previously described (Astorga-Eló et al., 2020). Fifty to 100 g of rhizosphere soils were placed into sterile plastic bags, sealed, and transported on ice to the Applied Microbial Ecology Laboratory (EMALAB) at La Frontera University, Temuco, La Araucanía, Chile.

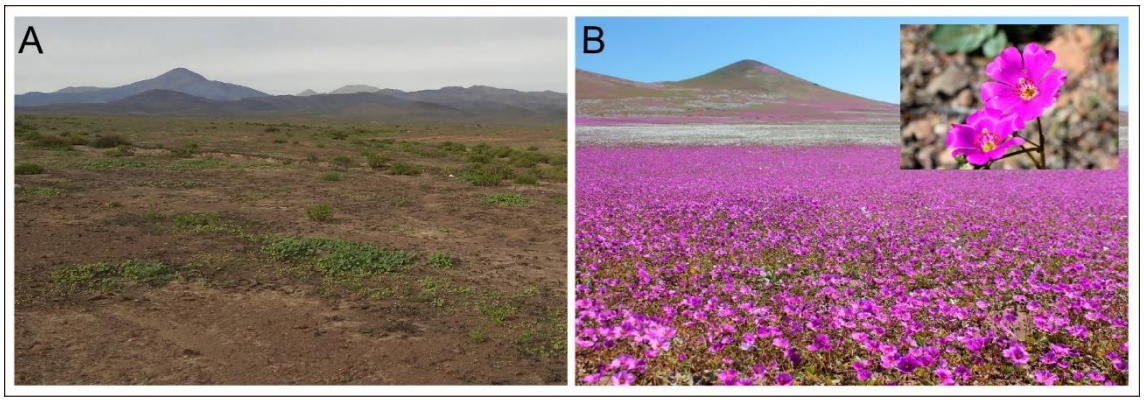

Figure 1. Mantles of *Cistanthe longiscapa* during pre-flowering (A) and full-flowering (B) stages at 2017 flowering desert event in Atacama Desert.

3.2 Isolation of culturable rhizobacterial strains

Culturable rhizobacteria from samples were obtained by platting serial dilutions of samples on different culture media (such Luria-Bertani (LB), NM-1 minimal medium and Tryptone Soya Agar (TSA) medium), and then by streaking the purified isolates on LB agar (Jorquera et al. 2014). Briefly, 10 g of rhizosphere sample were added to glass beaker containing 100 mL of sterile saline solution (0.8% NaCl). The suspension was vigorously shaken for 30 min at room temperature, serial dilutions were prepared in saline solution, and plated on LB agar plates. Plates were incubated for 4 days at 30°C. A total of 72 colonies with different phenotypes (color, elevation, edges, etc.) were isolated and purified by streaking onto LB agar plates. The purified isolated strains were stored at –80°C in 7:3 LB:Glycerol until needed for further analysis.

3.3 Determination of plant-growth promoting traits in rhizobacterial strains

The plant-growth promoting traits of the isolates were evaluated *in vitro* as follow. Phosphate solubilization (PS), was selected because Chilean soils have a great phosphorus retention capacity (Vásconez & Pinochet, 2018). PS activity was determined on agar plates using the National Botanical Research Institute's phosphate growth medium (NBRIP; 10 g l^{-1} D–glucose, 5 g l^{-1} Ca–phosphate, 5 g l^{-1} $MgCl_2 \times 6\ H_2O$, 0.25 g l^{-1} $MgSO_4 \times 7\ H_2O$, 0.2 g l^{-1} KCl, 0.1 g l^{-1} $(NH_4)_2SO_4$ and 15 g agar, according to Jorquera et al. (2008). The isolated strains were grown for 48 h at 30ºC and clear zones surrounding colonies was measured and taken as an indicator of PS activity. *Azospirillum/Herbaspirillum*-like rhizobacteria (N_2−fixing; NF) among isolated strains were screened by using NFb (nitrogen free broth) semi–solid culture medium, with malate as sole carbon source, as described by Baldani et al. (2014). The putative N_2–fixing rhizobacteria were revealed by a thin white growth near the top of the tubes. Inocula from these white zones were serially diluted in sterile 0.8% NaCl and plated onto Congo Red and Ashby's agar media for N_2–fixing bacteria previously described (Rodriguez-Caceres, 1982). Then, inocula from these white zones were taken, serially diluted (from 10^{-1} to 10^{-5} in sterile 0.8% NaCl) and plated on Red Congo agar and Ashby agar for N_2–fixing bacteria selection as selective culture media, where only N_2–fixing bacteria are able to growth (Rodriguez-Caceres, 1982).

Activity of 1–aminocyclopropane–1–carboxylate deaminase (ACCD), related with the ability to reduce the ethylene synthesis, was a feature selected since ethylene is produced by plants as response to damages or environmental stress (Zhang et al., 2020). ACCD in isolated strains was determined according to Penrose & Glick (2003), which measures the amount of α–ketobutyrate produced when ACCD cleaves the substrate

ACC. The amount of α–ketobutyrate produced (μmol) in each sample was determined by measuring absorbance at 540 nm using a MultiskanTM GO spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The A_{540nm} values obtained were compared to those of a standard curve made using pure α–ketobutyrate at concentrations ranging from 0.0 to 1.0 mmol L^{-1}. The ACCD activity is expressed as μmol α-ketobutyrate h^{-1} g protein. Production of tryptophan-induced auxins was determined and measured using the Salkowski colorimetric method, according to standard protocol described by Patten & Glick, (2002), where aliquots of bacterial culture were initially grown in DF salts minimal medium and transferred to DF salts minimal medium supplemented with 5 mM L^{-1} tryptophan. After incubation (36 h at 30°C) bacterial cells were removed from the cultures by centrifugation (3,000 × g) and supernatants were mixed vigorously with Salkowski's reagent (150 mL of concentrated H_2SO_4, 250 mL of distilled H_2O, 7.5 mL of 0.5 M $FeCl_3 \times 6H_2O$). Auxin content, primarily as indole acetic acid (IAA) was determined by measuring absorbance at 280 nm and by comparison to a standard curve produced using IAA at concentrations ranging from 0.0 to 50 μg mL^{-1}. Exopolysaccharides (EPS), responsible for biofilms formation, was analyzed using the acid hydrolysis method described by Parkar et al. (2001). One mL aliquots of 1% (w vol^{-1}) cold tryptophan were added to tubes and the samples were heated in a boiling bath for 20 min. After cooling, the amount of EPS produced was determined at A_{500nm} comparing the obtained values to a standard curve of glucose equivalents elaborated with pure sucrose at concentrations ranging from 0.0 to 5.0 mg mL^{-1} (Myszka & Czaczyk, 2009).

3.4 Compatibility of mixed cultures

The compatibility of the isolates was examined in order to assemble a rhizobacterial consortia. From the initial 72 isolated only 23 of them express some PGP trait, and were subjected to the compatibility test. Strains were genotyped based on partial sequencing of 16S rRNA genes using the universal primers set 27f (5'–AGA GTT TGA TCC TGG CTC AG–3') and 1492r (5'–TAC GGY TAC CTT GTT ACG ACT T–3') (Peace et al., 1994), and their growth compatibility was assayed on agar plates as described as Prasad & Babu (2017). Briefly, 50 µL aliquots of a cell suspension of each isolate was plated onto LB agar plates and incubated for 24 h at 30°C. After incubation, 5µL of each 23 isolates were deposited onto previously inoculated plates and re-incubated for 24 h at 30°C. Strain incompatibility was defined by the visual presence of inhibition of growth and isolates showing incompatibility or growth inhibition capability were discarded and not used for the formulation of consortia. After testing, only 12 isolates were able to grow without inhibiting the growth of other bacteria, thus eliminating most of the *Pseudomonas* strains, and then the strains identified as the same were eliminated. Finally, only 9 isolates were selected to formulate the consortia, which were considered different, regardless their taxonomic affiliation, for presenting different phenotypes (that is, expressing different PGP traits).

3.5 Assembly of rhizobacterial consortia

Based on results from plant growth-promoting (PGP) traits, genotyping, and compatibility tests, three rhizobacterial consortia were formulated using isolated strains belonging to *Bacillus*, *Paenibacillus* and *Brevibacillus* (phylum Firmicutes). Each selected strain was grown separately in LB broth, until a concentration of 10^6 CFU mL^{-1}. Later, equal quantities of each strain were obtained to assemble the respective consortia.

Each consortium was held at room temperature for 12 hours before being applied. all the three consortia were formulated to have more than three PGP traits (Table 1). Consortium "A" was composed of three strains relative to *Bacillus subtilis* providing 1 to 3 PGP traits, whereas consortium "B" was formulated with *B. subtilis, Paenibacillus polymyxa* and *Bacillus mojavensis,* showing three to five PGP traits, respectively. The consortium "C" was comprised of *B. subtilis*, *Bacillus altitudinis* and *Brevibacillus laterosporus*, showing only one PGP traits per isolate. This last consortium did not include isolated strains having exopolysaccharide production traits.

Table 1. Characterization of rhizobacterial isolates from *Cistanthe longiscapa* used in the formulation of consortia.

			Plant growth-promoting traits				
Consortium	**Isolate**	**Closest relatives or cloned sequences (accession no.)** †	**PS**	**NF**	**AU**	**ACC**	**EPS**
A	11	*Bacillus subtilis* BCRC 10255 (NR_116017)	–	+	–	+	+
	13	*Bacillus subtilis* BCRC 10255 (NR_116017)	+	–	–	–	+
	14	*Bacillus subtilis* BCRC 10255 (NR_116017)	–	–	+	–	–
B	17	*Bacillus subtilis* BCRC 10255 (NR_116017)	+	+	–	–	+
	3	*Paenibacillus polymyxa* DSM 36 (NR_ 117725)	+	+	+	+	+
	15	*Bacillus mojavensis* ifo 15718 (NR_118290)	+	+	+	+	+
C	14	*Bacillus subtilis* BCRC 10255 (NR_116017)	–	+	–	–	–
	4	*Bacillus altitudinis* BCZ2 (MF954002)	+	–	+	–	–
	12	*Brevibacillus laterosporus* DSM 25 (NR_112212)	–	–	–	+	–

† Based on partial sequencing of 16S rRNA gene and comparison with those present in GenBank by using BLASTn algorithm.

PS: phosphate solubilization; NF: Growth in N–free culture medium; AU: production of tryptophan–induced auxins; ACCD: 1–aminocyclopropane–1–carboxylate deaminase activity; EPS: production of exopolysaccharides.

3.6 Determination of plant-growth promoting traits in rhizobacterial consortia

While the plant growth-promoting PGP traits of individual strains were initially evaluated as described above, we wanted to determine if these same traits were maintained when strains were mixed together to form consortia. The plant growth-promoting traits were measured as described above, except for the production of tryptophan-induced auxins (AU) which was evaluated by high performance liquid chromatography (HPLC) according to the methods described by Lee et al. (2004). Briefly, overnight bacterial cultures (10 mL) were centrifugated at 3,000 × g and supernatants were filtered through 0.22 μm membranes to remove residual discard bacterial cells. Supernatants were analyzed by HPLC using a DAD Shimadzu, LC20AT pump, CTO 20AC furnace, and DAD SPD M20A detector, and a C18 reverse phase column (5 μm, 4.6 × 100 mm^{-2}). The mobile phase consisted of acetic acid (1.1%): acetonitrile (70:30) with a flow of 1 mL min^{-1}. The eluates were detected at 280 nm and auxins were identified and quantified by integration of the areas under the peaks using a standard curve prepared with pure IAA that was prepared using concentrations ranging from 0 to 75 μg mL^{-1}.

3.7 Plant inoculation assay with formulated rhizobacterial consortia

Plant growth-promotion by the formulated consortia was evaluated in inoculation assays done under greenhouse conditions using the standardized "Fundo El Vergel" (Angol city, Chile) protocol using tomato (*Lycopersicon esculentum* L., Cal Ace variety) as a plant model. Germination of 200 tomato seeds was evaluated once inoculated at pre- and post-sowing. For pre-sowed treatment, 100 seeds were submerged for 24 h in 20 mL of rhizobacterial suspension containing equal concentrations of each isolated at a total cell density of 10^6 CFU mL^{-1}. For post-sowing treatment, 100 seed were deposited into the

substrate and then inoculated with 0.2 mL of Inocula per seed (as equivalent for the pre-sowing treatment) (this procedure was the 1st inoculation). The percentage germination of seeds, by each consortium, was evaluated according to Tiquia & Tam (1998) and Schelin et al. (2003). Inoculated and non-inoculated control seeds (n=100) were sowed in pots using a commercial peat–based (90% vol vol^{-1} porosity and 75 kg m^{-3} of density) substrate and irrigated to 80% to 85% of field capacity (calculated as water holding capacity according to Nguyen et al., 2012) until germination in 8 to 20 d, where a 2nd inoculation was done with 2 mL of the rhizobacterial suspension at 10^6 CFU mL^{-1}. Every re-inoculation was done according to the change in the phenological stage of tomamto seedlings, not considering the number of days between every stage. The number of days between sowing and germination (germination time) was recorded for each treatment (n=100) and primary root length was measured from randomly selected plants (n=10). A 3rd inoculation was done at day 30 (as described above) when the seedlings showed first appearance of leaves (n=50). At day 40, a 4th inoculation was done, and seedlings were subjected to water scarcity stress (not irrigated) for 24, 72 and 120 h, in order to obtain smaller amounts of water available in the substrate. These periods of when water deficit was applied, were determined considering the minimum, and maximum, number of hours than a tomato seedling can survive without irrigation before the definitive transplanting, considering that maximum water consumption in tomato plant occurs during blooming and setting (Wu et al., 2021). Finally, 50 seedlings from each treatment were selected at day 45 for transplant into commercial coconut fiber and peat-based substrate (90% vol vol^{-1} porosity and 75 kg m^{-3} of density). After transplantation, plantlets were grown for 15 d and irrigated to 80% to 85% of field capacity. The percentage of plantlets (n=50) recovered after water stress, and plant height (cm), and fresh shoot weight (g) was measured at day 60 as previously described (Mayak et al., 2004; Mangmang et al., 2015).

The number of leaves developed by plants was measured after 20, 40 and 60 days, according to the phenological stage of the tomato plants.

For statistical analysis, the data obtained were subjected to one–way analysis of variance (ANOVA) and means were compared by the Tukey's test for multiple comparisons. Differences between treatments at $P \leq 0.05$ were considered to be significant.

4. Results

4.1 Assembly and determination of PGP traits of rhizobacterial consortia

Analyses of the partial sequences of 16S rRNA genes indicated that selected isolated strains belonged to the genera *Bacillus* (12), followed by *Pseudomonas* (6), *Brevibacillus* (4) and *Paenibacillus* (1). The Bacillus isolates had the greatest number of PGP traits, particularly the production of exopolysaccharides (EPS) and P solubilization (Fig. 2A). The production of tryptophan–induced auxins by selected isolated strains ranged from 0.1 to 15.6 μg of indole acetic acid mL^{-1} (Fig. 2B), the ACCD activity ranged from 10.6 to 15.1 of μmol of α–Ketobutyrate h^{-1} g $protein^{-1}$ (Fig. 2C), and the production of EPS ranged from 883 to 3667 μg of sucrose equivalents mL^{-1} of supernatant (Fig. 2D). When the compatibility of isolated strains was tested on agar plates, strains belonging to the genus *Pseudomonas* provoked growth inhibition of the other assayed strains, including *Pseudomonas* itself. Consequently, a decision was made of using only representatives of Firmicutes taxa for consortium assembly (Table 1). Initial mixture analyses indicated that the assembled consortia not only kept their PGP traits, but that some had higher PGP activities, with values of tryptophan–induced auxins production of indole acetic acid mL^{-1} increasing from 118.2 to 122.6 μg (a 3.7% increase), ACCD activity increasing from

27.1 to 68.7 µmol of α–Ketobutyrate h^{-1} g $protein^{-1}$ (a 253.5% increase), and EPS production increasing from 1085.3 to 3077.5 µg sucrose mL–1 supernatant (a 283.6% increase) (Table 2). It is noteworthy that the Consortium A, only formulated with B. subtilis strains, showed significantly ($P \leq 0.05$) higher values of PGP traits, and Consortium C showed an EPS production trait that was not observed when individual strains were characterized.

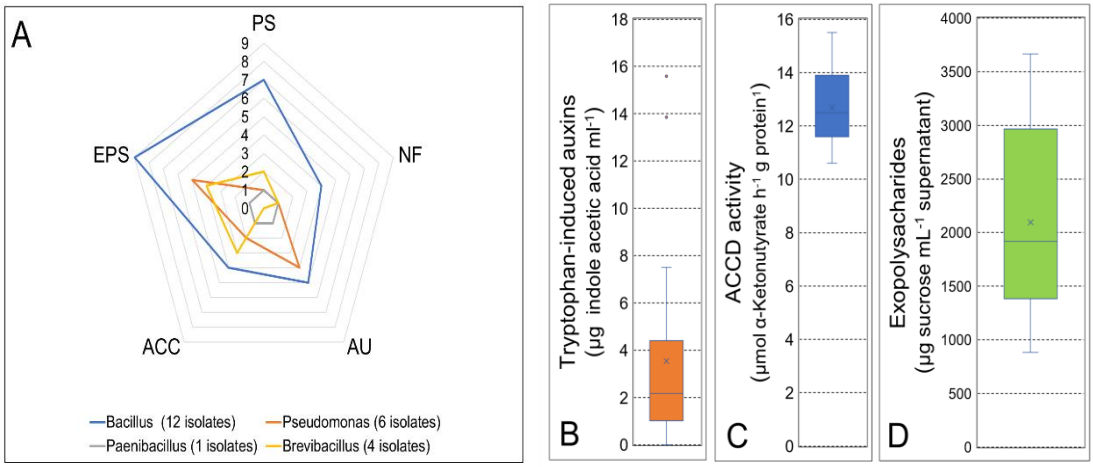

Figure 2. (**A**) Radial diagram showing the number of plant growth–promoting traits found in rhizobacterial strains isolated from *Cistanthe longiscapa*. PS: phosphate solubilization; NF: growth in N–free culture medium; AU: production of tryptophan–induced auxins (**B**); ACCD: 1–aminocyclopropane–1–carboxylate deaminase activity (**C**); EPS: production of exopolysaccharides (**D**).

Table 2. Plant growth–promoting traits in rhizobacterial consortia from *Cistanthe longiscapa.*

Consortium	Plant growth–promoting traits			
	PS (cm†)	**AU (μg IAA mL^{-1})**	**ACCD (μmol α–KB mg^{-1} protein)**	**EPS (μg sucrose mL^{-1} supernatant)**
A	1.80 ± 0.08* b‡	122.6 ± 8.98 a	68.7 ± 2.5 a	3077.5 ± 314.2 a
B	1.80 ± 0.07 b	118.2 ± 7.47 b	45.8 ± 3.9 b	3072.8 ±768.1 a
C	1.95 ± 0.05a	118.4 ± 6.46 b	27.1 ± 5.0 c	1085.3 ± 264.4 b

PS: phosphate solubilization; AU: production of tryptophan–induced auxins; ACCD: 1–aminocyclopropane–1–carboxylate deaminase activity; EPS: exopolysaccharides production; IAA: indole acetic acid; KB: α-ketobutyrate.

* Values represent the means ± standard deviation of *n*=3

† Ratio calculated as clear halo diameter / colony diameter of phosphate solubilization.

‡ Different letter in each column denotes significant differences ($P \leq 0.05$) by ANOVA followed by Tukey's post-hoc test.

4.2 Plant inoculation assay with formulated rhizobacterial consortia

The impact of formulated consortia on germination percent and germination time are presented in Table 3. Prior to sowing seeds inoculated with Consortium B showed a significant ($P \leq 0.05$) greater germination percentage (97%) compared with uninoculated controls (90%) and with inoculation with consortia A and C (90% and 92%, respectively). However, inoculation with consortium B also resulted in a significant greater ($P \leq 0.05$) germination time (12 d), relative to that seed with the control (18 d) and by inoculation with other consortia (9 and 10 d). When inoculation at sowing, seeds inoculated with Consortia B and C showed the significant ($P \leq 0.05$) higher germination rates (91% and 92%, respectively), compared with control (86%), and significantly ($P \leq 0.05$) shorter germination times (12, 15 and 10 d for consortia A, B and C, respectively) compared with control (18 d).

Table 3. Percentage and time of germination of tomato seeds inoculated with rhizobacterial consortia from *C. longiscapa* at pre–sowing and sowing.

Inoculation time	Consortium	Germination (%)	Germination time (days)
Pre–sowing	Control	90±5* b[†]	8±5 b
	A	90±5 b	9±5 b
	B	97±1 a	12±3 a
	C	92±2 b	10±3 a
Sowing	Control	86±8 b	18±2 a
	A	83±8 b	12±3 b
	B	91±2 a	15±4 b
	C	92±2 a	10±3 b

* Values represent the means± standard deviation of 50 seeds per treatment.

[†] Different letter in each column denotes significant differences ($P \leq 0.05$) by ANOVA followed by Tukey's post-hoc test.

Primary root length was measured after 15 d in all four treatments consisting of sowed seeds receiving consortia A, B and C and a control, prior to sowing or at sowing. Results in Table 4 show that seeds inoculated with consortia, at pre-sowing, obtained primary roots lengths shorter (3.1 to 3.5 cm), relative to the control treatment (5.5 to 5.6 cm). In contrast, plants inoculated with consortium B at sowing, had the longest primary roots (7.6 to 8.1 cm) and the C consortium inoculated treatment had similar primary root lengths in pre-sowing, and sowing treatments.

Table 4. Primary root lengths of 15-day-old tomato seedlings inoculates with formulated rhizobacterial consortia.

Inoculation time	**Consortium**	**Primary root lengths (cm)**
Pre–sowing	Control	5.5 ± 0.5* b[†]
	A	3.1 ± 0.5 c
	B	7.6 ± 0.2 a
	C	6.8 ± 0.2 a
Sowing	Control	5.6 ± 0.3 c
	A	3.8 ± 0.2 d
	B	8.1 ± 0.3 a
	C	6.9 ± 0.2 b

* Values represent the means± standard deviation of 10 seeds per treatment.

[†] Different letter in each column denotes significant differences ($P \leq 0.05$) by ANOVA followed by Tukey's post-hoc test.

The impact of no irrigation on plantlets recovery, growth, and number of leaves developed by seedlings was also evaluated and the results are summarized in Table 5. All the inoculated plants (100%) survived 24 h without irrigation, while uninoculated control

plants only had 92% recovery. There was a dramatic and significant ($P \leq 0.05$) effect of inoculation on plant recovery after 72 h without irrigation and plants inoculated with Consortium A had 100% recovery, compared with controls with 67% recovery. Moreover, even after 120 h of no irrigation, the inoculated plants showed significantly ($P \leq 0.05$) greater recovery (from 88% to 96%), compared with the control (54%).

The beneficial effect of the consortia on tomato plants was also evidenced 15 d after emergence, particularly in seedlings subjected to no irrigation treatments for 72 h and 120 h (Table 5). Inoculated plants exposed to 24 h of water scarcity showed higher heights (averaging from 54.8 to 59.4 cm) compared to uninoculated controls (average 51.3 cm). Similar effects were obtained for fresh shoot weight, and significantly greater weights were seen in seedlings inoculated with Consortium A and C (200.4 g and 214.7 g, respectively), compared with uninoculated controls (197.7 g). At 72 h of no irrigation, the three consortia resulted in plants with heights close to 60 cm, which was statistically higher ($P \leq 0.05$) compared to control (41.5 cm). Significantly greater weights ($P \leq 0.05$) were also obtained in plants inoculated with Consortium A (198.5 g) and C (195.8 g), compared with control plants (178.7 g). A similar trend was observed at 120 h of no irrigation, where plants had significantly ($P \leq 0.05$) greater heights (from 48.5 to 52.8 cm) and weights (from 167.5 to 175.3 g) were obtained in plants inoculated with rhizobacterial consortia compared with those without inoculation (37.9 cm and 146.9 g). Generally, plants inoculated with Consortium A had better recovery from water stress, whereas plants inoculated with Consortium C showed better growth.

Table 5. Recovery and growth of tomato seedlings inoculated with rhizobacterial consortia and exposed to no irrigation for different time intervals (24, 72 and 120 h) prior to transplant to definitive substrate

No irrigation time (h)	Consortium	Recovery (%)	Height (cm)*	Weight (g)*	Number of leaves (days after transplant)		
					20 d	40 d	60 d
24 h	Control	92 ± 5** b†	51.3 ± 5.3 b	197.7 ± 8.9 b	4.1 ± 0.2 b	5.1 ± 0.1 d	6.8 ± 0.2 a
	A	100 ± 1 a	59.4 ± 4.2 a	200.4 ± 9.8 b	4.9 ± 0.1 a	5.6 ± 0.1 c	6.1 ± 0.3 b
	B	100 ± 1 a	58.7 ± 4.1 a	189.8 ± 8.6 b	4.0 ± 0.2 c	6.1 ± 0.2 b	6.9 ± 0.2 a
	C	100 ± 1 a	54.8 ± 3.5 b	214.7 ± 7.3 a	3.5 ± 0.3 d	6.5 ± 0.1 a	7.1 ± 0.1 a
72 h	Control	67 ± 10 c	41.5 ± 3.7 b	178.7 ± 9.8 c	3.2 ± 0.1 a	5.1 ± 0.3 b	5.9 ± 0.1 c
	A	100 ± 1a	58.8 ± 4.3 a	198.5 ± 4.5 a	3.9 ± 0.2 a	5.4 ± 0.2 b	6.5 ± 0.1 b
	B	92 ± 5 b	59.5 ± 5.2 a	187.3 ± 6.2 b	2.9 ± 0.1 c	5.9 ± 0.2 a	6.8 ± 0.1 a
	C	92 ± 5 b	60.1 ± 3.7 a	195.8 ± 5.7 a	4.2 ± 0.1 a	6.1 ± 0.1 a	6.5 ± 0.2 b
120 h	Control	54 ± 10 c	37.3 ± 5.3 c	146.9 ± 8.3 c	3.0 ± 0.2 a	4.5 ± 0.1 c	4.7 ± 0.1 c
	A	96 ± 2 a	48.5 ± 3.2 b	167.5 ± 6.5 b	2.7 ± 0.2 b	4.9 ± 0.2 a	5.7 ± 0.2 b
	B	88 ± 5 b	51.3 ± 4.9 b	171.1 ± 7.2 a	2.5 ± 0.3 b	4.8 ± 0.3 b	5.8 ± 0.1 b
	C	92 ± 3 b	52.8 ± 2.2 a	175.3 ± 6.3 a	3.1 ± 0.2 a	5.3 ± 0.2 a	6.3 ± 0.1 a

* Growth was measured after 60 days since transplant.

** Values are means± standard deviation of 50 seedlings per treatment.

† Different letters in the column denote significant differences ($P \leq 0.05$) by ANOVA followed by Tukey's post-hoc test.

The number of leaves formed after transplanting was used as an indicator of impacts due to water stress (Table 5). Seedlings receiving 24 h of water stress, showed no great differences after inoculation with consortia A, B or C, with control plants 20 days after transplanting. Similarly, after 60 days of transplanting the differences in leaf number was not significant. Seedlings receiving 72 h of water scarcity, started with the same patterns as did seedlings with 24 h of stress. Inoculation of plants with consortium B had the least effect on leaf development, after 20 days of recovery. After 60 days, however, results seen with plants receiving consortium B were similar to those receiving consortia A and C, and to the control treatment. The 120-h water stress treatment similarly affected leaf numbers on all plants after 20, 40 and 60 days of recovery. However, the effect of inoculation on leaf number was significantly than that seen with the control (more than 6 to 7 leaves developed in 60 days versus 4 to 5 leaves in the control). In addition, inoculation with Consortium C allowed plants to develop more leaves in the same period. Inoculation with consortia A and B had a positive effect on leaves, but lower than that seen with consortium C.

5. Discussion

Our study revealed the occurrence of several bacterial strains with plant growth-promoting traits in rhizosphere of *C. longiscapa* during a FD event in the Atacama Desert, a few studied subject so far. The strains were taxonomically affiliated to members of the genera *Bacillus*, *Paenibacillus*, *Brevibacillus*, and *Pseudomonas*. Diverse studies have revealed the presence of potential PGPR associated with plants in arid ecosystems, including Atacama Desert (Jorquera et al., 2014; Inostroza et al., 2017). Both *Bacillus* and *Pseudomonas* spp. strains showing growth promoting traits are commonly used as

soil inoculants and recognized by their resilience to harsh desert conditions (Santoyo et al., 2012; El-Sayed et al., 2014). Similarly, members of *Paenibacillus* and *Brevibacillus* have also been found in the rhizosphere of desert plants and proposed to be used as PGPR (Soussi et al., 2016).

The production of EPS was the main PGPR trait found in isolated strains. EPS has long been recognized as providing important benefits to microbiota, either living as single organisms, in binary associations, or in heterogeneous mixed communities in adverse environments (Wolfaardt et al., 1999). It has been proposed that EPS functions as protect microorganisms from ultraviolet radiation, extreme temperature, extreme pH, high salinity, high pressure, poor nutrients, among others harsh features found in extreme environments (Yin et al., 2019). EPS has also been shown to protect the plant–associated rhizobacteria from water stress by enhancing water retention and by enhancing root colonization and attachment by the formation of a network of fibrillar material that permanently connects the cells to the root surface (Marvasi et al., 2010). It should be noted that our isolated strains produced greater amount of EPS compared with *Bacillus* and *Paenibacillus* isolates found among rhizobacteria from desert plants (Kavamura et al., 2013). In addition, *Bacillus* enhanced EPS production capacity has been used to increased soil moisture when maize was grown under drought stress (Khan et al., 2017).

Rhizobacterial *Bacillus* strains with auxin production and ACCD deaminase activity have also been isolated from plants growing in arid environments (Kumar et al., 2012; Chari et al., 2018). The quantity of tryptophan–induced auxins produced by our isolated strains were lower (from 0.1 to 15.6 μg mL^{-1}) than those observed in rhizobacteria different from *Bacillus* in desert soils, but similar to those in the same environment (from 23 to 37 for non-*Bacillus* bacteria, and 4.2 to 9.2 g mL^{-1} for *Bacillus* species) by Goswami et al. (2015). In contrast, ACCD production by our isolated strains was much (10.6 to 15.1 of

μmol of α–Ketobutyrate h^{-1} g $protein^{-1}$) than those reported by reported by Acuña et al. (2019) in rhizobacteria from native plants grown in Andean altiplano of the Atacama Desert (0.8 to 3.3 μmol of α–ketobutyrate h^{-1} g $protein^{-1}$). However, these amounts are lower than other strains (4.6 from *B. licheniformis* K11 to 402.1 from *Enterobacter cloacae*) isolated from plants under drought conditions (Lim & Kim 2013; Danish et al., 2020).

We also showed that some strains are compatible between them and these make possible to assemble a consortia of plant growth-promoting rhizobacteria. Based on the results from compatibility tests, our consortia were only formulated using member of the phylum Firmicutes. While individual strains have been reported to be PGPR, we expected synergistic or enhanced activity in each formulated consortium (Paerl & Pinckney, 1996; Kumar et al., 2016). This idea was confirmed in our study, where all assayed consortia not only kept their individual PGP traits (e.g., ability to growth in N-free culture medium), but also had enhanced ACCD activity compared with those values obtained for individual isolated strains. In addition, while there was a trend towards greater production of tryptophan–induced auxins in the consortia compared with individual cells, we cannot directly compare results as different methods were used to quantify auxins in individual cells (Salkowsky reagent) vs consortia (HPLC). Interestingly, Consortium C, which was formulated with isolated strains that individually did not show EPS production, showed EPS production when strains were mixed together. This is likely due to general synergistic effects that allow members of Consortium C to optimize use of resources (Khan et al., 2017). In addition, a protective effect of EPS on rhizobacteria under inhospitable conditions has also been observed by Alami et al. (2000) and plants treated with EPS–producing rhizobacteria also display an increased resistance to water stress (Naseem et al., 2018; Nadeem et al., 2020). It is known that EPS provide a

microenvironment that holds water and dries up more slowly than the surrounding environment protecting to bacteria and plant roots against desiccation (Selvakumar et al., 2012).

In relation to the tomato inoculation assay with formulated rhizobacterial consortia, the application of consortium, at sowing and pre–sowing, resulted in significant ($P \leq 0.05$) higher germination percentage and less time required for seed germination, in seeds inoculated at sowing, compared with uninoculated controls. Since germination is mediated by ethylene production (Pluskota et al., 2019), and Consotium "A" was the high ACCD activity, the reduced germination percentage and the number of days to germinate would be associated to this feature. This was particularly evident in seeds inoculated with Consortium B and C. Addition of PGPR strains has been previously shown to increase seed germination and facilitated production of healthy seedling (Prasad et al., 2017). Inoculated seeds, pre-sowing, took more days to germinate than did control treatment. Despite this, inoculation of tomato with any of our consortia resulted in greater germination percentages, especially by the B consortium. Considering that Consortium A had the greatest production of AU, and this is related with seeds germination, at least as shown for soybean (Shuai et al., 2017), the greater production of this phytohormone could repress seed germination as we can see using consortia B and C, which had significantly higher germination percentage than did plants inoculated with Consortium A and the control.

When tomato seedlings were subjected to different period of stress by no irrigation, our results revealed significantly ($P \leq 0.05$) higher percentage of recovery in seedlings inoculated with the rhizobacterial consortia, compared with uninoculated controls. Similarly, despite that a significant effect of rhizobacteria consortia on plant growth was not observed in seedlings exposed to 24 h of water scarcity stress, inoculation

with the three formulated consortia resulted in significantly greater growth in plants when longer periods of no irrigation were applied (72 h and 120 h). Vurukonda et al. (2016) indicated that physiological changes in plants induced by microorganisms results in enhanced tolerance to drought stresses by PGP mechanisms, including EPS, auxin production and ACCD activity. The ACCD activity is recognized as a main PGP mechanism to ameliorate the abiotic stress in plants (Glick, 2004), and numerous studies have demonstrated that ACCD–producing rhizobacteria can increase the fresh and dry weights of tomato and pepper seedlings and reduce the ethylene production under drought stress (Lingwy ey al., 2016). Since transplanting shock results in plant growth retardation and developmental delay, and sometimes seedling death (Dong et al., 2020), the application of a PGP consortium would help to ameliorate this negative effect. Furthermore, in lettuce nursery, using bacterial bioestimulants, the transplant shock was reduced compared to a control treatment (Vetrano et al., 2020).

Primary root length after germination was used as indicator of plant capacity to explore substrate and to reach nutrients and water from soil. As shown by our results, excessive AU production can explain the shorter primary root measured in plants receiving Consortium A (Shuai et al., 2017). In contrast, B and C consortia showed similar AU production, but lower than that seen with the A consortium. Interestingly, the primary root length of plants under the influence of B and C consortia showed longer primary roots. These results imply a better establishments and further seedling shoot growth and development of tomato plants.

As the main photosynthetic organs, leaves are important for an optimum growth and development of plants. In tomato production, the endurance of plantlets is strictly related to the number and turgor of leaves. As shown by our results, plants subjected to the consortium effect developed more leaves as they were under more days of stress by

water scarcity. The cumulative effect of PGP traits from the consortium would explain these observations. The main production of ACCD, especially for Consortium A, exerted a positive development of plants under water stress, as Moustaine et al. (2017) describe. Seedlings commonly experience temporary periods of abiotic stresses, resulting in necessary root and shoot metabolic and structural adjustments to withstand stress conditions. As described by Cordero et al. (2018), inoculation of seedlings with PGPR can improve the recovery and development of plants to stresses such as water scarcity, drought, high salt content and others. In our results, consortia B and C show the most promising results in recovery and growth of tomato seedlings, compared to control.

6. Conclusions

This current study revealed the occurrence of culturable rhizobacteria (mostly *Bacillus* and *Pseudomonas*) harbouring PGP traits in the rhizosphere of *C. longiscapa* growing during a FD event. Moreover, based on their taxonomic affiliation, compatibility tests and PGP traits, we successfully formulated three rhizobacterial consortia, which improved seed germination, recovery and growth of tomato seedlings exposed to no irrigation stress under commercial greenhouse conditions. In this sense, desert plants are an important source of PGPR having all the traits for thrive in extreme conditions. Therefore, the application of isolated native rhizobacteria adapted to arid conditions could be a valuable tool to improve the growth, and tolerance, of vegetables under water scarcity as results of drier and harsher environment under climate change scenarios. This is especially true for the production of seedlings which are under pronounced stress periods during transport from production to the field and the use of PGP consortia could improve the plantlets recovery to transplant shock. Further studies on the interactions between components of autochthonous soil and rhizosphere microbiota are necessary in order to

understand bacterial assemblages. The study of the potential of PGPR isolated from desert plants represent a special interest to validate their use at a commercial scale. This information may contribute to development of an agricultural system that is less dependent on water availability.

7. References

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CHAPTER IV

General discussion and conclusions

4.1 GENERAL DISCUSSION

The Flowering Desert phenomenon, at the Atacama Desert, Chile, is an unexpected event, where multiple life forms emerge when it occurs. Most of the common viewers are focused on the magnitude of visible features such as multiple-colored flowers blooming (Vargas et al., 2019), butterflies, worms, snails, birds, and some reptiles (Vidiella et al., 1999) that induce people to forget the changes in the extreme environment they are observing, but under the soil surface there is more activity than in the upper part.

As reported in the first publication of this Doctoral Thesis, the Atacama Desert is inhabited predominantly by Proteobacteria and Actinobacteria during the Flowering Desert (FD) event, followed by Chloroflexi and Gemmatimonadetes, as previously reported as dominant phyla in the rhizosphere of native Atacama Desert plants (Jorquera et al., 2014, 2016). Being bacteria able to thrive in hyperarid soils with extremely low organic C and N levels suggests that their abundance in arid soils implies that they are important colonists (DeBruyn et al., 2011). This is important because in the search for plant growth-promoting rhizobacteria (PGPR) from arid environments, *Bacillus* and *Pseudomonas* species are recognized as principal PGPR and belong to the most represented phylum found in FD. Since root exudates modify soil properties, and soil properties can produce qualitative and quantitative changes in exudate composition (Vives-Peris et al., 2020), mayor represented bacteria are able to be recruited by plants, but this recruitment is based on the capabilities of bacteria. If PGPR are present, more is the possibility of rhizobacteria being recruited according to the changes in root exudates depending on the phenological plant stage trying their best to recruit beneficial microbes by root exudates when they are needed (Yuan et al., 2018). The rhizomicrobiome is deeply influenced on plant growth, and the rhizomicrobiome assemblage and plant growth-associated microbes are differentially structured by soil properties, also, soil

microorganisms are more sensitive indicators to short-term changes chemical soil properties (Xiao et al., 2017: Hermans et al., 2020) than changes in root exudates, providing biologically relevant insights on the changes in individual soil parameters.

To obtain the best representatives of PGPR with the ability to enhance the resistance of plants to harsh environmental conditions (Dimkpa et al., 2009; Kumar & Verma, 2018), rhizobacteria from plants in extreme environments, such as studied in the first specific objective, are the best suitable candidates (Araya et al., 2020; Gaete et al, 2020). Evidence shows that PGPR from an extreme environment are most fitted to conditions considered as extreme (high UV-radiation, low water availability, high temperatures, high salinity of the soil, among others). With this genetical advantage, PGPR from desert plants are, as an example, naturally capable of thriving in places where 'normal' microorganisms cannot compete or cannot develop adaptations in short periods of time. Nevertheless, the rhizobacteria from *C. longiscapa*, thrive in a modified and transient environment, as FD is, since the greater availability of water triggering the FD event, must be able to grow and establish themselves in the rhizosphere in short periods of time, and contribute to the growth and development of *C. longiscapa*, under optimal conditions for this FD plant. However, this environment would be challenging for crops that grow in non-desertic climates.

As described in this research, some extreme PGPR can produce exopolysaccharides (EPS), or biofilms that generate a microenvironment in plant roots promoting not only beneficial traits to plants, but also a carbon source to microorganisms from the plants root exudates (Banerjee et al., 2019). EPS can reduce evaporation and increase soil water retention (Zheng et al., 2018), which is a crucial function considering that water scarcity is the main dominant parameter in desert zones. With the presence of EPS producing PGPR, vegetable production in a water limitation scenario, would

contribute to improve the drought tolerance of plants (Nadeem et al., 2020). In addition, rhizobacteria producing EPS, are able to mitigate effects against salinity and water pressure by enhancing the soil structure (Abbas et al., 2019). Furthermore, EPS containing Na^+ cations adhered beneficiates plants by let the Na^+ cations inaccessible to plants in saline environment (Timmusk et al., 2014), such as shown in the published article from this Thesis, where Na was influencing the microbial community during the 2017 FD event, helping to mitigate the possible saline stress that the plants would face.

In desert ecosystems, such as the studied in this research, is possible to find a great diversity of bacteria (Contador et al., 2020), most of them identified only at the genomic level (16S rDNA) but, as the publication has shown (Astorga-Eló et al., 2020), the underrepresented bacteria are the most related to predominant groups. This "microbial dark matter" has enormous potential that, even with the difficulties of studying it, shows its great importance in the interrelationships established within bacterial communities (Andrei et al., 2017; Bernard et al., 2018). Our results reveal the relevance of minor taxa (or rare taxa) impacting rhizosphere processes for fast growth of native plants during FD, one of the most extraordinary, and scarcely studied, natural events in Atacama Desert, one of the driest places in the globe.

In Chile, seedlings and plantlets production requires less surface for their establishment, but there are serious issues to face, including seasonality, special infrastructure, good quality water availability, protection against diseases, control of plagues, germination substrates, and commercial varieties most required by the market (Flaño, 2014), and transplant shock, where seedling are lost due to adaptations problems to the new environment, outside the orchards . In the second and third specific objectives of this Doctoral Thesis, the aim was to select, culture and isolate PGPR from the FD and analysed their effect in plantlets under different periods of no irrigation stress.

Nevertheless, it is necessary highlight that salinity tolerance in our isolates was not considered, but further studies focused on to determine their salinity tolerance, and tolerance to drought or water scarcity effects, would be an important approach to investigating rhizobacteria from AD.

Commonly the logistic parameters in the plantlets production are not considered, meaning that transport conditions, acclimatization of plantlets, transplant shock or unforeseen changes from the germination substrates to field conditions are responsible for the most losses in the vegetable production (Özer, 2018), being often necessary replanting, increasing the final costs. To address these important concerns, our work was focused on plantlets recovery, imitating water scarcity from seedling production to field cropping. Our results demonstrated that consortia of PGPR from Atacama Desert FD events represent a feasible tool to protect tomato plantlets from water scarcity stress, as rhizobacterial consortia evaluated as demonstrated as well (Guo et al., 2019), allowing tomato plantlets to recover from the water stress once irrigation was re-established after transplantation. Safeguard plantlets against environmental stresses will become a strategy focusing in to avoid losses, diminishing production costs and, at the long term, increasing the production and yield of the vegetables.

One alternative to achieving these significant issues is applying all the knowledge obtained so far into field practices. Plantlets produced in some places would be cultivated in different locations, even it is possible that destination would have limited water availability in which cases the treatment of seeds or seedlings with beneficial microorganisms, such as PGPR, could be a major factor to consider for protecting plantlets of stressing conditions derived from transport or handling (Ansari et al., 2017). Thus, inoculation of seeds with PGPR have demonstrated to be a good alternative in this matter, not only diminish the loss of seedlings, but also by improving the nutrient

acquisition and decreasing the presence of plagues (Hernández-Montiel et al., 2017). If treatment for control of wilting in plantlets is implemented, it would be a very effective alternative to control plantlet loss, especially in places where water scarcity and high temperatures are experienced, as our results suggest.

Information about taxonomic classification, PGP traits and laboratory conditions performance of these bacteria are available in most publications, reviews, and books (Kumari et al., 2019), but there is no equal amount of information about their effect on field conditions or considering a big extension of crops. Much of the technical information it is in saline soils, because of the desert cultivation techniques, involves increasing of salt levels at root zones as response of the water scarcity. However, scarce information is available on plants growing in other conditions (Ansari et al., 2017), indicating a lack of research infrastructure to perform such investigations, no association with large-scale producers, or miscommunication between researchers and producers. In addition, bacterial assemblages have been reported as strategies that benefit the thrive and fitness of plants. These groups of bacteria called consortia, can help to cope with multiple stressors depending on what PGP trait has each bacterium (Rajput et al., 2018). Furthermore, if every bacterium from these consortia has different PGP features or the combination of bacteria promotes synergism between them, the benefit for plants, o vegetable production, increased, as well the protection between the rhizobacteria forming the consortium (Rana et al., 2012; Jha & Saraf, 2012).

Another alternative addressing this issue is the format of PGPR transference and application. Even though information about genetic potential, laboratory performance, little scale vegetable production of PGPR were known, their transition from an extreme environment, which is associated with a particular plant, it must be able to be replicated successfully in a new environment and associated with a different plant species (Alsharif

et al., 2020; Zhang et al., 2019). Besides, the compatibility between extreme foreign PGPR and native bacteria must be considered. Thereby, if the process of isolation, characterization of extreme PGPR succeed, there is the problem of propagation and application in the field. Because of the few publications about PGPR tested in the field, there is scare information about the number of bacteria needed, the best way for their manipulation and transportation, and their inoculation in soils. Much of the studies occupied bacterial solutions in liquid forms, which is a concern about the volume transported to the field (Polanco & Betancourt, 2000). Furthermore, sometimes it is not thought of as a regular agricultural practice (Martínez et al., 2010), because of the risk associated with the use of microorganisms, which is mainly associated with cultural issues than scientific evidence so far, especially in our country, where technologies of so-called biofertilizers have been imported from foreign countries. However, there is no a legislation about it, increasing the low acceptance of this PGPR in cultures (Pagnani et al., 2020). In other cases, bacteria are applied to soil as powder inoculant in seeds (Kloeper et al., 1981). However, the main concern is tracking the new bacteria introduced into the soil (Rilling et al., 2019) and determine if their beneficial effect is prologued in time, no matter how they were introduced in soils (Ahmad et al., 2011).

4.2. GENERAL CONCLUSIONS

Our results show that the microbial diversity of rhizobacteria from *C. longiscapa* during the Flowering Desert event at the Atacama Desert, changes between pre-flowering and full flowering stages of the plant. However, there are no apparent variations from one flowering desert event to another, indicating that diversity of bacteria associated with this phenomenon are adapted to this representative plant and their unique environment.

Changes in rhizobacterial abundance and diversity could be explained by the phenological stage of the native plant (which translates into the variation of the root exudates) and, to a lesser extent, by the climatic conditions.

In addition, it is possible to conclude that some rhizobacteria associated with *C. longiscapa* have the ability necessary to be considered as PGPR, having different PGP traits such as P-solubilization, N_2 fixation, ACC-deaminase, IAA, and EPS production. In order to obtain all these PGP capacities, it is possible to assemble a group of compatible rhizobacteria, with different PGP traits, forming a consortium, where every bacterium is able to express one, or more traits, helping not only to plants coping harsh environmental stressors, but helping to each other to thrive in such conditions. Even more, consortia are able to express, sometimes, PGP traits not expressed individually, as shown in the results of the experiments for the 2nd and 3rd specific objectives in this research. Every rhizobacterium isolated, mainly belonging to *Bacillus* and *Pseudomonas* genus, were able to express these traits under laboratory conditions. In addition, assemblies of these bacteria, excluding *Pseudomonas*, maintained the PGP traits, while others expressed characteristics that, individually, were not present, indicating a synergistic activity between the grouped bacteria artificial assemblages. Inoculated to tomato seeds, these rhizobacterial consortia assembled from isolated obtained from rhizosphere of *C. longiscapa* during 2017 FD event, were able to transfer their beneficial PGP traits to tomato seedling, especially those where consortium "C" was applied, allowing them to thrive to the water scarcity stress, or improving the detrimental effects of transplant shock.

With these results, and considering the hypothesis raised in this thesis, it is possible to accept it, since it was possible to isolate rhizobacteria from *C. longiscapa*, from the Flowering Desert, with plant growth promoting capacity. Besides, it was possible to

assemble consortia from them and to determine that these beneficial characteristics were transferred to plants subjected to stress by water scarcity.

4.3. FUTURE DIRECTIONS

Climate change is undoubtedly affecting the climate in Chile, as well as other agricultural countries. Under this scenario, there is an enormous potential for research and utilization of PGPR in cropping, especially considering the presence of multiple extreme environments in our country. Nevertheless, the search for extreme PGPR with the ability to help horticultural crops cope with environmental stressors has to be limited to prospection, isolation, and identification. In this research, consortium formulated with Firmicutes representatives which. This recommendation is based in the results of plant recovery, leaves developed, and the ability to produce EPS once rhizobacteria were assembled. If further research are focused in corroborate the presence of all the isolated in the inocula solution (as example, using MALDI-TOF, molecular techniques or flow cytometry) it would be a tool to improve crop production under water scarcity scenario, ensuring the presence of rhizobacteria. In addition, it must include testing the PGP traits in the field, focusing in to evaluate under real water stress, the potential effect of PGPR. Aiming future researchers to the investigation on the field or associated with vegetable producers. This associations will help improve crops and help them cope with changing climate conditions. They would contribute to generating knowledge in producers about the benefits of using beneficial bacteria in crops, helping to improve legislation on the use of biofertilizers. In this sense, the mechanisms of how beneficial bacteria contribute to the adaptation of plants are necessary by itself but and must go hand in hand with the study of the environmental factors to which crops are subjected.

Under the scenario that the availability of PGPR allows the production of mass-use products, there should be investigations intended to determine the best way to store and distribute these bacteria in the field, either as individual bacteria or as consortia, since having them and knowing their capabilities is not enough when it comes to distributing them for mass use. Being the freeze-dried the best method for preserve the PGPR before their use, it would be important to analyze the use of liquid solutions, powder solutions or micro, or nano, encapsulated bacteria as an alternative to apply the selected PGPR in a short period of time covering the individual characteristics of field. This would be a good research focus if scientists could be linked more closely with the producers.

In another perspective, the role of the least represented microorganisms (known as microbial "dark matter") is still little known and, to date, the existence of most of them is only known through high-throughput sequencing techniques. With new sequencing techniques, which are increasingly advanced, it will be possible to identify many more microorganisms. However, by itself, this information will not be of great use if analysis systems are not developed to identify their metabolic potential and the role they play within the microbiological community in which they are present.

Considering the presence of various biomes and environments in our country, all biological resources, including the diversity of PGPR, should be used under a patrimonial perspective in order to improve current agricultural systems, promoting the use of microorganisms, or assemblages of them, specially selected to counteract adverse environmental effects and favor a greater production of food, and sustainability in its production.

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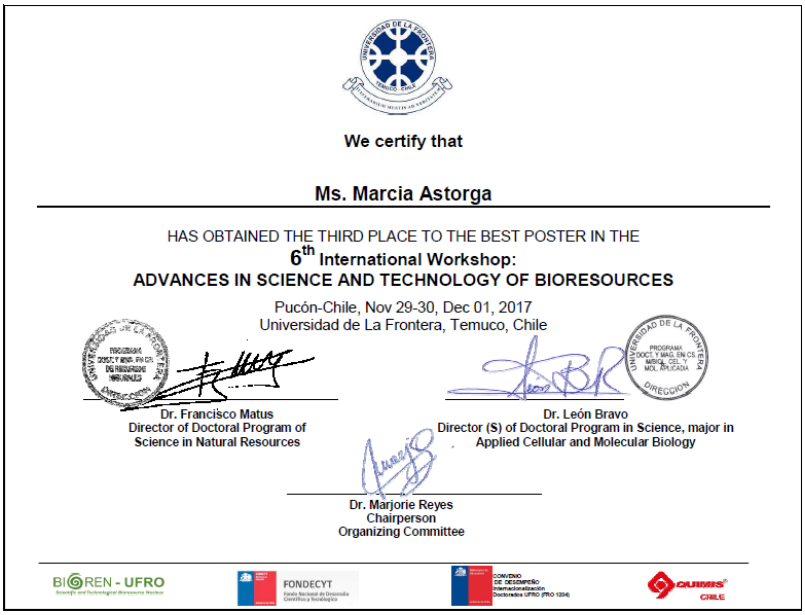
ANNEXES

Annex 1.

Award for Best Poster in the VI International Workshop: Advances in science and technology of bioresources (Pucón 2017, Chile). Poster entitled "Composition of bacterial communities associated with C*isthante sp*. rhizosphere during Flowering Desert phenomenon in the Atacama Desert, Chile".



We certify that

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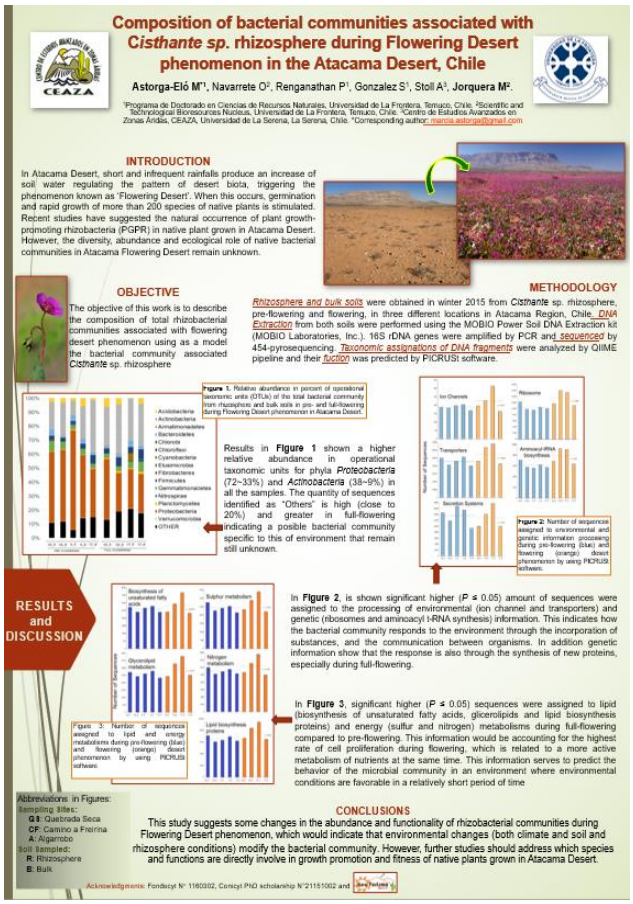

Composition of bacterial communities associated with *Cisthante sp.* rhizosphere during Flowering Desert phenomenon in the Atacama Desert, Chile

Astorga-Eló M[1*], Navarrete O[2], Renganathan P[1], Gonzalez S[1], Stoll A[3], Jorquera M[2].

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INTRODUCTION

In Atacama Desert, short and infrequent rainfalls produce an increase of soil water regulating the pattern of desert biota, triggering the phenomenon known as 'Flowering Desert'. When this occurs, germination and rapid growth of more than 200 species of native plants is stimulated. Recent studies have suggested the natural occurrence of plant growth-promoting rhizobacteria (PGPR) in native plant grown in Atacama Desert. However, the diversity, abundance and ecological role of native bacterial communities in Atacama Flowering Desert remain unknown.

OBJECTIVE

The objective of this work is to describe the composition of total rhizobacterial communities associated with flowering desert phenomenon using as a model the bacterial community associated *Cisthante* sp. rhizosphere

METHODOLOGY

Rhizosphere and bulk soils were obtained in winter 2015 from *Cisthante* sp. rhizosphere, pre-flowering and flowering, in three different locations in Atacama Region, Chile. *DNA Extraction* from both soils were performed using the MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories, Inc.). 16S rDNA genes were amplified by PCR and *sequenced* by 454-pyrosequencing. *Taxonomic assignations of DNA fragments* were analyzed by QIIME pipeline and their *fuction* was predicted by PICRUSt software.

Figure 1. Relative abundance in percent of operational taxonomic units (OTUs) of the total bacterial community from rhizosphere and bulk soils in pre- and full-flowering during Flowering Desert phenomenon in Atacama Desert.

Results in **Figure 1** shown a higher relative abundance in operational taxonomic units for phyla *Proteobacteria* (72–33%) and *Actinobacteria* (38–9%) in all the samples. The quantity of sequences identified as "Others" is high (close to 20%) and greater in full-flowering indicating a posible bacterial community specific to this of environment that remain still unknown.

Figure 2: Number of sequences assigned to environmental and genetic information processing during pre-flowering (blue) and flowering (orange) desert phenomenon by using PICRUSt software.

RESULTS and DISCUSSION

In **Figure 2**, is shown significant higher ($P \leq 0.05$) amount of sequences were assigned to the processing of environmental (ion channel and transporters) and genetic (ribosomes and aminoacyl t-RNA synthesis) information. This indicates how the bacterial community responds to the environment through the incorporation of substances, and the communication between organisms. In addition genetic information show that the response is also through the synthesis of new proteins, especially during full-flowering.

Figure 3: Number of sequences assigned to lipid and energy metabolisms during pre-flowering (blue) and flowering (orange) desert phenomenon by using PICRUSt software.

In **Figure 3**, significant higher ($P \leq 0.05$) sequences were assigned to lipid (biosynthesis of unsaturated fatty acids, glicerolipids and lipid biosynthesis proteins) and energy (sulfur and nitrogen) metabolisms during full-flowering compared to pre-flowering. This information would be accounting for the highest rate of cell proliferation during flowering, which is related to a more active metabolism of nutrients at the same time. This information serves to predict the behavior of the microbial community in an environment where environmental conditions are favorable in a relatively short period of time

Abbreviations in Figures:
Sampling Sites:
QS: Quebrada Seca
CF: Camino a Freirina
A: Algarrobo
Soil Sampled:
R: Rhizosphere
B: Bulk

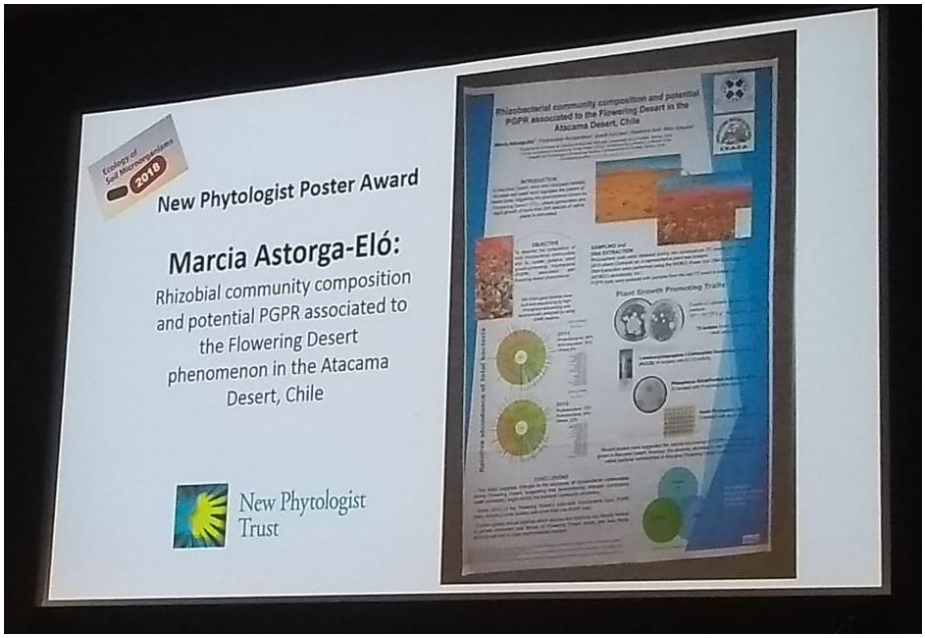
CONCLUSIONS

This study suggests some changes in the abundance and functionality of rhizobacterial communities during Flowering Desert phenomenon, which would indicate that environmental changes (both climate and soil and rhizosphere conditions) modify the bacterial community. However, further studies should address which species and functions are directly involve in growth promotion and fitness of native plants grown in Atacama Desert.

Acknowledgments: Fondecyt N° 1160302, Concyt PhD scholarship N°21151002 and

Annex 2.

New Phytologist Poster Award in the III Conference on the Ecology of Soil Microorganisms (ESM 2018, Helsinki, Finland). Poster entitled “Rhizobial community composition and potential PGPR associated to the Flowering Desert phenomenon un the Atacama Desert, Chile.”



Annex 3.

Assistance Scholarship from the Organizing committee of the I ISME Latin America Congress (Valparaiso 2019, Chile). Poster entitled “Rhizobial community composition and potential PGPR associated to the Flowering Desert phenomenon un the Atacama Desert, Chile.”

Gmail — Marcia Astorga <marcia.astorga@gmail.com>

Beca Primer Congreso ISME América Latina

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La beca consiste en la suma de clp 385.500.- (Trescientos Ochenta y Cinco Mil Quinientos pesos chilenos) lo cual equivale a 500 euros. Esta suma le será entregada, en efectivo, en pesos chilenos, al momento de su registro el miércoles 11 de septiembre de 2019.

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Saludos cordiales,

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Primer Congreso ISME América Latina
11-13 septiembre 2019
Universidad Técnica Federico Santa María
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Annex 4.

Published paper corresponding to objective 1 as described in these Thesis. Available at https://www.frontiersin.org/articles/10.3389/fmicb.2020.00571/full?report=reader

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ORIGINAL RESEARCH
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Composition, Predicted Functions and Co-occurrence Networks of Rhizobacterial Communities Impacting Flowering Desert Events in the Atacama Desert, Chile

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Flowering desert (FD) events consist of the rapid flowering of a wide variety of native plants in the Atacama Desert of Chile, which is categorized as the driest desert in the world. While ephemeral plants are an integral part of the desert ecosystem, there is little knowledge on plant-microbe interactions that occur during FD events. Consequently, the overall goals of this present study were to investigate changes in the composition and potential functions of rhizobacterial community of *Cistanthe longiscapa* (Montiaceae) during the 2014 and 2015 FD events and determine the composition, potential functions, and co-occurrence networks of rhizobacterial community associated with the root zone of *C. longiscapa* during pre- (PF) and full-flowering (FF) phenological stages. Results of this study showed that the Proteobacteria and Actinobacteria were the dominant taxa in rhizosphere soils during the three FD events (2014, 2015, and 2017) examined. In general, greater microbial richness and diversity were observed in rhizosphere soils during the 2015-, compared with the 2014-FD event. Similarly, predicted functional analyses indicated that a larger number of sequences were assigned to information processing (e.g., ion channel, transporters and ribosome) and metabolism (e.g., lipids, nitrogen, and sulfur) during 2015 compared with 2014. Despite the lack of significant differences in diversity among PF and FF stages, the combined analysis of rhizobacterial community data, along with data concerning rhizosphere soil properties, evidenced differences among both phenological stages and suggested that sodium is a relevant abiotic factor shaping the rhizosphere. In general, no significant differences in predicted functions (most of them assigned to chemoheterotrophy, magnesium metabolisms, and fermentation) were observed among PF and FF. Co-occurrence analysis revealed the complex rhizobacterial interactions that

Annex 5.

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