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Endophytic bacteria of the rock-dwelling cactus *Mammillaria fraileana* affect plant growth and mobilization of elements from rocks

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This study is dedicated for the memory of the German/Spanish mycorrhizae researcher Dr. Horst Vierheilig (1964–2011) of CSIC, Spain.

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ABSTRACT

Mammillaria fraileana is a major pioneer, small cactus that harbors endophytic bacteria that have plant growth-promoting traits, including rock-weathering capacity. Our working hypothesis was that this functional group of endophytic bacteria assists in establishing pioneer plants on rocks. When these endophytic bacteria were inoculated on seedlings grown in rock substrate, mobilization of elements from the substrate increased at variable levels across combinations of substrates and inoculants. In plants grown in the rhyodacite substrate, where these cacti naturally grow, increased mobilization occurred in plants inoculated with several strains. Promotion of plant growth, manifested as an increase in dry weight, was greater in cacti inoculated with *Enterobacter sakazakii* M2PFe. Accumulation of nocturnal acids, indicating photosynthesis by crassulacean acid metabolism, was superior in plants inoculated with the endophytes *Azotobacter vinelandii* M2Per and *Pseudomonas putida* M5TSA. Inoculation with endophytes can stimulate plant growth of *M. fraileana* by mobilizing elements from rock, which can lead to higher photosynthetic activity and accumulation of biomass. Inoculation with *P. putida* M5TSA also led to accumulation of more total nitrogen than plants inoculated with a control nitrogen-fixing bacteria. Evidence of endophytic colonization is provided after initial inoculation of seedlings and re-isolation and sequencing of 16S DNA of recovered bacteria from developing disinfected plants. The associative interaction between pioneer cacti and their bacterial endophytes enable the host plants to grow in places where plants do not normally grow. Through colonization and establishment of pioneer plants, soil is created, which facilitates colonization by other desert species and contributes to the diversity of dry lands.

1. Introduction

Plant-mediated interactions are receiving greater attention for their importance on structuring multi-trophic above and below ground communities, since these interactions are highly diverse and involve all phyla (van Dam and Heil, 2011). Plant-microbial interactions can be classified into three basic groups: (1) negative (pathogenic) interactions; (2) positive interactions, in which either both partners derive benefits from close association (mutualism) and both partners derive benefits from loose association or only one partner derives benefits without harming the other (associative); and (3) neutral interactions, where none of the partners derives a direct benefit from interaction and neither is harmed (Singh et al., 2004). Among associative interactions, endophytism can be regarded as synergistic interactions benefiting both organisms (Hardoim et al., 2008; Ryan et al., 2008). It is now established that almost all plants are colonized by a diverse array of endophytic microorganisms (Strobel, 2007; Sturz and Nowak, 2000). Microbial endophytes (bacteria, fungi, and AM fungi) are defined as microorganisms that colonize internal tissues of plants and show no external signs of negative effect (Danhorn and Fuqua, 2007; Ryan et al., 2008; Schulz and Boyle, 2006).

The effect of bacterial endophytes on plant performance was mainly studied in crops. Certain endophytes have been shown to: (a) Increase plant resistance to pathogens by production of phytoalexins, accumulation of pathogenesis-related proteins, and deposition of structural barriers (Benhamou et al., 2000; Kloepper and Ryu, 2006); (b) Control insect pests (Azevedo et al., 2000; Fahey et al., 1991) and pathogenic nematodes (Hallmann et al., 1998) by production of toxic compounds (antibiosis) in the host plant; and (c) Improve plant growth in general (Hardoim et al., 2008; Sturz et al., 1997). Furthermore, plant–bacterial endophyte associations

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have potential for improving phytoremediation of soils by increasing degradation of pollutants, such as herbicides (Germaine et al., 2006) and increasing heavy metal uptake and translocation in hyper-accumulating plants (Doty, 2008). In addition to enhancing the ability of plants to tolerate pollutants in their tissues, endophytes can metabolize some contaminants inside plants (Doty, 2008). Commercial applications of bacterial endophytes are inoculants in agriculture (Sturz and Nowak, 2000) and a source of secondary metabolites for medical applications (Strobel, 2007).

Promotion of plant growth mediated by endophytic bacteria may be exerted by several mechanisms, such as production and regulation of levels of phytohormones, including indole-3-acetic acid and ethylene (Hardoim et al., 2008; Long et al., 2008), nitrogen fixation (Doty et al., 2009), synthesis of siderophores (Ramesh et al., 2009), and solubilization of minerals, including phosphorus (Puente et al., 2009a; Vessey, 2003). Mechanisms for mineral solubilization by the plant–endophytic bacteria association remain unclear, but may result from secreting short-chain organic acids of element-specific ligands that are able to change the pH of the substrate and enhance chelation (Carrillo et al., 2002; Vessey, 2003). As a consequence, minerals dissolved outside of plants result in increased mobilization of elements into the plants (Puente et al., 2004a). Bacterial endophytes can also increase photosynthetic activity (Chi et al., 2005), accelerate emergence of seedlings, and promote establishment of plants under adverse conditions (Forchetti et al., 2010; Puente et al., 2009b). All these positive traits notwithstanding, activity of endophytic bacteria in wild plants is scarcely studied.

In some arid regions of Mexico, plants such as cacti commonly establish on rocks without soil (Bashan et al., 2002, 2006b; Lopez et al., 2009; Valverde et al., 2004). These plants can create associative interactions with mycorrhizal fungi and rhizoplane bacteria and their growth is enhanced by inoculation with these bacteria (Bashan et al., 2007; Puente et al., 2004a,b). Endophytic bacteria colonizing roots of the giant cardon *Pachycereus pringlei* cactus were shown to be associated with rock weathering, which in turn supplies cacti with inorganic nutrients and fixed nitrogen (Puente et al., 2009a,b).

In this study, we investigated the cactus *Mammillaria fraileana* and its endophytes. This small cactus grows in clusters of individual plants. They have narrow cylindrical stems (3-cm diameter, up to 30 cm high), pink flowers, and red fruits that contain numerous small black seeds (Fig. 1A; Anderson, 2001). This endemic cactus is common on rocky habitats along the east coast of the southern part of the Baja California Peninsula (Wiggins, 1980). In an earlier study, we found that *M. fraileana* is the main plant species colonizing

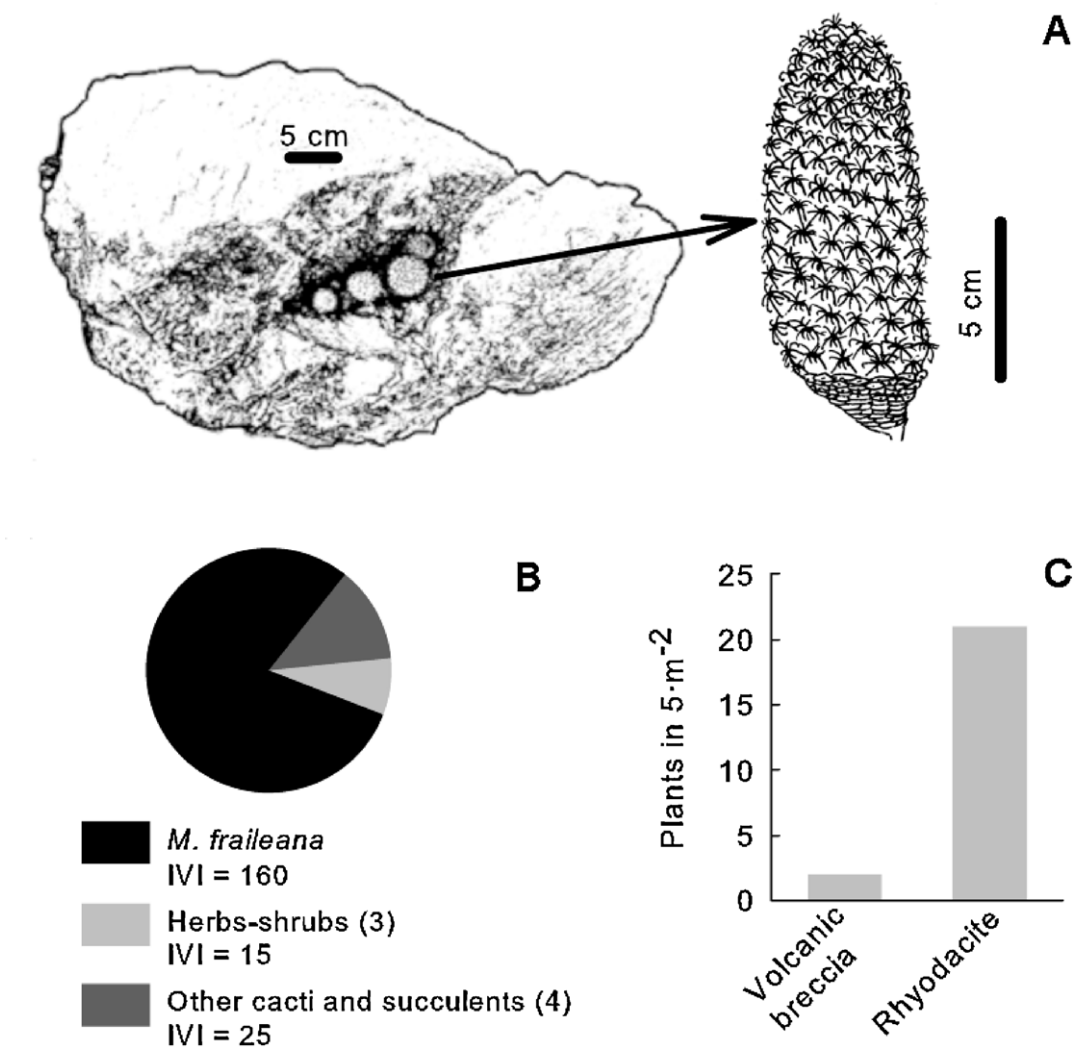

Fig. 1. Ecological importance of the rock-colonizing cactus *Mammillaria fraileana*. (A) Plants growing in rock fissures without soil, (B) distribution of the abundance of plants colonizing bare rocks. Ecological importance in terms of Importance Value Index (IVI). Maximum value for IVI = 200. In parenthesis, the number of species counted for each group of plants, (C) variation of abundance of *M. fraileana* recorded in two types of rocks. Data modified from Lopez et al. (2009).

rocks ranging from rhyodacite to rhyolite (from ancient volcanic eruptions), and that the mineral composition and type of rock influence the abundance of this cactus (Fig. 1B and C; Lopez et al., 2009). Plants can be found in rock fissures or on the rock surface without the presence of soil (Fig. 1A; Lopez et al., 2009). Because rhyodacite is weathered to a greater extent in the presence of these cacti, it is assumed that this cactus is directly associated with large-scale, rock weathering and formation of soil (Bashan et al., 2002; Lopez et al., 2011). Yet, there is essentially no evidence to support this claim.

In a recent study, we isolated several endophytic bacteria with the capacity to fix nitrogen, degrade rhyodacite, and solubilize phosphate *in vitro* (Lopez et al., 2011). Our working hypothesis in this study was that this group of bacteria, having potential plant growth-promoting traits, participates in mobilizing minerals in rocks, and assists in establishing these cacti on rocks. This hypothesis was tested by studying how mobilization of elements and several parameters of plant growth are affected by inoculation with endophytic bacteria and the type of substrate these plants use in nature.

2. Materials and methods

2.1. Organisms

Mammillaria fraileana (Britt. and Rose, 1923) Boedeker (local name 'viejito' or 'little old man') was used in all experiments. Plants were inoculated with four strains of endophytic bacteria previously isolated from roots of *M. fraileana* growing on rock (Lopez et al., 2011): *Bacillus megaterium* M1PCa, *Enterobacter sakazakii* M2PFe, *Pseudomonas putida*, and *Azotobacter vinelandii* M2Per (GenBank accession numbers: GQ504713, GQ504715, GQ504714, and GQ504712, respectively). These strains were tested as plant growth-promoting bacteria (PGPB) because they showed potential traits for PGPB, such as fixing nitrogen, solubilizing minerals, and weathering rock *in vitro* (Lopez et al., 2011). Endophytic bacteria were tested separately and as a consortium of the four strains. We used two positive PGPB controls, *Azospirillum brasilense* Cd (ATCC 29710) and *Bacillus pumilus* ES4 (GenBank accession number FJ032017), isolated from the giant cardon cactus *P. pringlei*. The cactus and its bacteria are known to weather rock (Puente et al., 2006, 2009b). The negative control was cacti that were not inoculated.

2.2. Substrates

Three different substrates for growing plants were used: (1) Pulverized rhyodacite + perlite (RPE, 1:8, w/w) served as the tested substrate and representing the substrate where *M. fraileana* grows plentifully in nature. (See Lopez et al., 2009 for chemical composition of these rocks.) Perlite (Supreme Perlite, Portland, OR) was added to reduce the bulk density of the ground rock to allow plants to grow; (2) The positive substrate control (PNS), intended to provide sufficient nutrients to plants and consisting of perlite (<0.53–0.85 μm at proportions described below) supplemented with 1/10 Hoagland's nutrient solution during watering (complete formula in mg L^{-1}): 492 $MgSO_4$, 1.81 $MnCl_2{\cdot}4H_2O$, 2.86 H_3BO_3, 0.22 $ZnSO_4{\cdot}5H_2O$, 0.078 $CuSO4{\cdot}7H_2O$, 0.12 $NaMoO_4{\cdot}H_2O$, 1.8 $CaCl_2$, 16 KSO_4, 0.5 K_2HPO_4, 100 KNO_3, 3 $Ca(NO_3){\cdot}4H_2O$, and 0.06 mL 0.5% $FeSO_4$ + 0.4% tartaric acid); (3) The negative substrate control (PE) represented the substrate that is low in nutrients and consisting only of perlite.

To produce the RPE substrate for the greenhouse experiment, ten fragments of rhyodacite (~250 g each) were collected from a ridge in Baja California Sur, Mexico (24°11′15″N, 110°17′50″W), pulverized, and sieved to 74-μm particle size (200 screen mesh). The substrate in each pot (3.5 cm × 3.5 cm × 6 cm high, 9 g substrate) was prepared by adding 1 g pulverized rock to multi-sized perlite: 3 g perlite (0.85–1.4 μm), 4 g perlite (0.53–0.85 μm), 1 g perlite (<0.53 μm) (1:8 ratio). For positive and negative controls, the perlite substrate was similar, but 1 g perlite (<0.53 μm) was substituted for rock powder. For the micro-growth chamber experiment, perlite (<1 mm particle size) was used (Thermo-o-Rock, West, AZ, USA) and the ratio in RPE was 1:6 (w/w).

2.3. Plant growth conditions and inoculation with endophytic strains

2.3.1. Experiment in micro-growth chamber

The response of plants grown on RPE was measured after inoculation with two endophytic bacteria and two bacterial controls. Endophytic bacteria *P. putida* M5TSA and *A. vinelandii* M2Per were considered putative PGPB. The positive control PGPB was *A. brasilense* Cd and the negative control consisted of plants that were not inoculated.

The substrate was sterilized by autoclave and aseptically placed in large, sterile, polystyrene square Petri dishes (24 cm × 24 cm × 18 mm; #351040, BD Biosciences, San Jose, CA) that serve as micro-growth chambers for these small seedlings. The surface of batches of 0.36 g of seeds (~2300 seeds) was disinfected through a 5-step procedure: Pre-treatment with 2% Tween-20 for 30 min with mechanical agitation; 10 rinses with sterile tap water; surface sterilization with 1.5% (v/v) 6% NaOCl solution with mechanical agitation for 5 min; 10 rinses with sterile, distilled water; and immersion for 2–3 s in 100% ethanol. The seeds were then air-dried under a laminar flow hood. Success of sterilization procedure was verified by plating rinsing water from the last rinse on soy trypticase agar (TSA; Difco-BBL, Sparks, MD) and incubating the plates for 10 days at 35 ± 1 °C. Substrate was irrigated with deionized water to saturation and each batch of seeds was spread directly on the substrate. Petri dishes were partly sealed with parafilm (to allow gas diffusion) and then incubated in growth chambers (Conviron 125 and Conviron CMP, Winnipeg, MB, Canada) at 25 ± 1 °C at 55% relative humidity during a 6:18 day–night photoperiod. In a previous study (Lopez et al., 2011), low light intensity of 10 ± 2 μmol m^{-2} s^{-1} was sufficient to germinate seeds and sprout seedlings. Seedlings were inoculated one-month after germination. Aliquots of 4 mL (in 0.002 M potassium phosphate buffer, PBSK, pH 7.2) of washed bacterial suspension at a final concentration of 1×10^9 CFU mL^{-1} were uniformly spread on the substrate with the seeds. For a negative control, 4 mL PBSK were added to the substrate. Sterile water was aseptically provided every 15 days. The experiment was carried out in six replicates, where two Petri dishes served as a replicate and each contained ~1300 seedlings.

2.3.2. Experiment in the greenhouse

Seeds were surface-sterilized through the 5-step procedure described in Section 2.3.1. All other materials in this section were sterilized by autoclave or surface-sterilized by swabbing or spraying with 70% ethanol. Success of sterilization procedure was verified, as above, using groups of 100 seeds. Disinfected seeds were germinated on 1% nutrient agar and incubated in a growth chamber for 2.5 months (Model 815, Precision Scientific, Chicago, IL) at 30 ± 0.1 °C, 80% relative humidity, 8:16 day–night photoperiod, 10 ± 1 μmol m^{-2} s^{-1} light intensity. Two-month-old seedlings without bacterial contamination of the agar were aseptically transferred to small plastic pots containing each of the three substrates described above. The pots were individually placed into small sterile units made with clear plastic and domed lids to create a microenvironment with adequate space for gas diffusion. These units were created to minimize contamination from external

bacteria. The plants in these units were grown in greenhouse under the following conditions: day temperature (25 ± 5 °C), night temperature (17 ± 5 °C, 80% relative humidity, 11:13 day–night photoperiod, 340–370 μmol photon m^{-2} s^{-1} light intensity. After acclimation for 15 days, each pot was inoculated with 1 mL bacterial suspension in PBSK at a concentration of 1×10^9 CFU mL^{-1} of one of the following: *B. megaterium* M1PCa, *E. sakazakii* M2PFe, *P. putida* M5TSA, *A. vinelandii* M2Per, a consortium of the above endophytes, or two controls (*A. brasilense* Cd or *Bacillus pumillus* ES4). The negative controls were watered only with sterile, distilled water. Pots were irrigated every 15 days by adding 7 mL sterile, distilled water to substrates RPE and PE (negative control) or an equal volume of sterile 10% Hoagland's nutrient solution to the positive control (PNS). This experiment lasted 8 months. The experiment was carried out in four replicates where pots (two) serve as a replicate and each pot contained 20 seedlings. This experiment also served for measuring biomass, enumerating endophytic bacteria, and determining the amount of 13 elements in the plants.

2.4. Enumerating culturable endophytic populations within plants after inoculation

Plants from the greenhouse experiment were aseptically harvested 3.5 months after inoculation. A composite sample of five plants was obtained from each treatment of the seven bacterial inoculants (including positive controls) and from the three substrates that were assayed ($n = 18$). Each composite of five plants was surface-sterilized in a four-step procedure: 35 min at moderate agitation with 1:1 (v/v) 2% chloramine and 2% Tween-20; five rinses with sterile, deionized water; immersion in 70% ethanol for 30 s; and a final quick rinse with 10 mL deionized water. Then, the plants were placed on sterile filter paper and air-dried for 5 min in a laminar hood chamber. Each composite of five plants per treatment were weighed and then macerated in 0.5 mL PBSK buffer with a glass pestle with frosted grinding surface. Serial dilutions of the fresh, macerated material were plated on general purpose trypticase soy agar (TSA) or on Rennie N-free (Rennie, 1981) medium only for plants inoculated with the nitrogen-fixing strain *A. vinelandii* M2Per. Plates were incubated at 30 ± 1 °C for 48–72 h and then counted for colony-forming units. Three replicates for each treatment were plated; each replicate consisted of three plates.

2.5. Production of biomass

At 5.5 months after inoculation, all plants were harvested and counted. The substrate was removed from the roots by gently soaking the plants in sterile deionized water. Dry weight was obtained by oven-drying (Thelco 31478, GCA Precision Scientific, Chicago, IL, USA) at 60 °C for 5 days. Four replicates represented each treatment and each replicate consisted of two pots with 11–20 plants per pot.

2.6. Determination of element content of plants

For determining biomass, the dried plants were placed in Eppendorf tubes and then ground to a fine powder using a glass pestle with frosted grinding surface. Small amounts of powder (~10 mg) were glued to specimen mount holders. For X-ray microanalyses, samples of the powder were observed with a scanning electron microscope (S-3000N, Hitachi High-Technologies, Tokyo, Japan) fitted with an X-ray detector (7021 with Inca 2000, Oxford Instruments, Scotts Valley, CA, USA). The working conditions were: 20 kV of accelerating voltage, 15 mm working distance, 100× magnification, and 800 μm × 1200 μm × 3 μm depth for surface analysis. Quantitative determinations for 13 elements (C, N, Ca, Mg, K, Na, P, Fe, Cu, Mn, Al, Zn, Si) were measured with three iterations per sample. Data for all elements was normalized by the equipment and expressed as percentage of weight. Four replicates were used for the analysis of each substrate.

2.7. Effect of endophytic bacteria on nitrogen content of plants grown in micro-growth chamber

Nitrogen content was determined using plants harvested 4 months after inoculation. Six samples per treatment were collected (~1500 plants per sample). Substrate was removed from the plants by gently soaking and rinsing with distilled water. Plants were dried in an oven at 55 °C for 75 h (Hafo 1680, Sheldon Manufacturing, Cornelius, OR, USA). Dried plants were ground to coarse granular size with a steel micro-spatula and then analyzed for total nitrogen content by automatic microKjeldahl (Digestion System 12.1009 and Kjeltec Auto 1030 Analyzer, Tecator, Höganäs, Sweden). Each sample was analyzed in triplicate.

2.8. Acid level measurements of plants grown in micro-growth chamber

At 5.5 months after inoculation, entire plants (~5 mm height) were harvested at 06:00 h and 18:00 h. Six replicates of each treatment were collected; each consisted of plants randomly selected (~25 plants per sample). Particles of substrate were carefully removed from the plant roots with dissecting tweezers. Plants were first weighed together to reach 0.2 g^{-1} fresh weight, then placed in Eppendorf tubes containing 1 mL 60% ethyl alcohol and stored at −20 °C until the assay of acidity. Concentration of total organic acids was measured according to Zotz and Andrade (1998), with the following modifications: Plants of each sample were macerated by hand with a glass homogenizer (Bellco Glass, Vineland, NJ), then the homogenate was brought to a volume of 20 mL with 60% ethyl alcohol, filtered by vacuum at 15–20 cm Hg, and boiled for 6 min. When cool, the sample was titrated to an endpoint of pH 7.0 using 0.010 N NaOH. Results of titration were expressed as total acid concentration in mmol H^+ g^{-1} fresh weight of plants. Nocturnal acid accumulation expressed the nocturnal increase of acidity (dawn value minus the dusk value) (Nobel and Berry, 1985).

2.9. Sequencing of 16S DNA of culturable bacteria re-isolated from plants after inoculation

One month after inoculation of seedlings, culturable bacteria were recovered from disinfected plants. Bacterial DNA was extracted and 16S DNA gene was amplified with the universal primers for Eubacteria 27 F and 1495R. The 16S sequencing was performed using a 3130 genetic analyzer sequencer (Applied Biosystems-Hitachi, Japan). Details are in supplementary materials. Identification of the recovered isolates was done by comparison with sequences of the original strains previously deposited in the GenBank database or by best match, using the BLAST tool (www.ncbi.nlm.nih.gov/Blast.cgi).

2.10. Statistics

The average values of bacterial counts were analyzed as $\log_{10}$ CFU g^{-1} dry weight of each replicate. Averaging content of N and increase of nocturnal acidity were analyzed for each replicate. The above variables were analyzed by one-way ANOVA, followed by ad-hoc Tukey–Kramer HSD analysis. Dry weight determination could not be done on individual small plants because they weighed a few mg (dry weight) per plant; therefore, dry weight was designated as the dry weight of 1000 plants. Data of the relative abundance of elements was first arcsine-transformed before statistical analysis. Dry weight and abundance of elements were analyzed by two-way ANOVA for overall effect of substrate (source

of element), inocula and for interactive effects of these two variables. Further analyses by one-way ANOVA were done for simple effects of inoculation treatments on the content of elements or dry biomass. All ANOVA and Tukey–Kramer HSD tests were evaluated at $P < 0.05$ using JMP 8.0 statistical software (SAS Institute, 2008).

3. Results

3.1. Populations of endophytic bacteria after inoculation and identification by 16S DNA

At 3.5 months after inoculation, the level of culturable endophytic bacteria in plants, on a dry weight basis, ranged from $6.37 \pm 0 \log_{10}$ CFU g^{-1} to $8.66 \pm 0.05 \log_{10}$ CFU g^{-1} (equivalent to 83.3×10^3 to 16.55×10^6 CFU g^{-1} fresh weight). Regardless of the species of bacteria, for plants grown on RPE and PNS, populations of endophytic bacteria varied and depended on the substrates ($P < 0.05$). For all substrates, the highest population was detected in plants inoculated with *P. putida* M5TSA ($8.66 \pm 0.05 \log_{10}$ CFU g^{-1} dry weight). This was followed by the treatment of inoculation with the positive control (*A. brasilense* Cd) and inoculation with the consortium of endophytes (Fig. 2A). Contrary to expectations, culturable endophytic bacteria of untreated plants were high and at levels (dry weight basis) that ranged from $6.47 \pm 0.05 \log_{10}$ CFU g^{-1} to $6.93 \pm 0.05 \log_{10}$ CFU g^{-1}. These populations were comparable to populations in treatments inoculated with endophytic bacteria *B. megaterium* M1PCa, *E. sakazakii* M2PFe, and *A. vinelandii* M2Per (Fig. 2A).

In a second experiment after inoculation of seedlings, subsequent plant growth, and recovery of endophytes from plants, analysis of the 16S DNA of the recovered bacteria confirmed the presence of the endophytes *B. megaterium* M1PCa, *E. sakazakii* M2PFe, and *P. putida* M5TSA, alone and in the consortium. The two bacteria used as positive controls, *A. brasilense* Cd and *B. pumilus* ES4, were also detected. In the untreated plants, only *Methylobacterium* sp. was recovered, while *Achromobacter xyloxidans* was detected occasionally in inoculated plants (Table S1, sequences are provided as supplementary material).

3.2. Biomass

Two-way ANOVA indicated that there was no overall effect of substrate ($F_{2,69} = 2.77$, $P > 0.05$) or inoculation ($F_{7,69} = 0.98$, $P > 0.05$) on dry weight of plants; however, the interaction between substrate × inoculation was significant ($F_{14,69} = 2.26$, $P < 0.01$). Since the performance of the inoculation treatments may vary depending on the substrate in which the plants grew, we searched for simple effects within each substrate. Plants grown on PE, deficient in nutrients, did not respond to the inoculation treatments ($F_{7,22} = 1.29$, $P > 0.05$, Fig. 2B), while the dry weight of plants grown on RPE, the native substrate ($F_{7,23} = 2.51$, $P < 0.04$, Fig. 2B) and PNS, substrate supplemented with nutrients ($F_{7,22} = 3.02$, $P < 0.02$, Fig. 2B) varied significantly among inoculation treatments. For plants grown on RPE, the non-inoculated treatment (0.69 ± 0.12 g g^{-1} for 1000 plants, Fig. 2B) had the lowest dry weight; the highest dry weight was in plants inoculated with *E. sakazakii* M2PFe (1.4 ± 0.14 g g^{-1} for 1000 plants, Fig. 2B). In plants grown on PNS, the dry weight increased only in the treatment with the endophyte *P. putida* M5TSA, which had the highest dry weight among all treatments (1.5 ± 0.13 g g^{-1} for 1000 plants, Fig. 2B).

3.3. Element content of inoculated plants

To determine variations in the amount of elements in young plants of *M. fraileana* growing on their native rhyodacite (RPE) substrate and inoculated with endophytic bacteria, we used three substrates: RPE, PE (negative control deficient in nutrients), and PNS (positive control supplemented with nutrients). The amount of elements represented their mobilization as a consequence of the effect of substrate and inoculants. For the average amount of each element in plants growing in the three substrates, C and N were the main constituents of plants (~45% and ~41%, respectively). In trailing order, the values were Zn (~5%), Na (~3%), Si (~2%); Ca, K, and Al varied (~1%); and P, Mg, and Fe were all below 0.5%. Cu and Mn were present in trace amounts (Table 1).

Two-way ANOVA shows the effect by substrate, inoculum, and the interaction substrate × inoculum on the abundance of elements in plants (F values at $P < 0.05$ are in Table 1). The effect of inoculum was significant for all the elements measured. Most of the elements, except Zn and P, were affected by the type of substrate. For the effect of substrate × inoculum, only C, Mg, and Fe were not influenced by this interaction (Table 1). General trends in the abundance of elements in the three substrates could not be defined; however, an overall effect of inoculum, mainly by endophytes, was detected (Table 2). Higher amounts of Al, K, Mg, and Si where found in plants inoculated with *P. putida* M5TSA. *A. vinelandii* M2Per led to high contents of C and Fe in plants, whereas the highest contents of Ca and P where measured in plants inoculated with *B. megaterium* M1PCa or *P. putida* M5TSA. Only N increased by inoculation with the positive control *B. pumilus* ES4. Untreated plants had higher amounts of Zn (Table 2).

Since we intended to determine variations in the amount of elements in plants growing on the native substrate of *M. fraileana* (rhyodacite; RPE), we analyzed the effect of inoculation on plants growing on RPE and considered the two control substrates as references. In general, the effect of inoculation on the content of Mg, Ca, N, Na, and P in plants grown in RPE was similar to the overall pattern in the three substrates (Table 2, Fig. 3).

In plants grown in RPE, most of the inoculation treatments increased the content of C and only plants inoculated with *B. megaterium* M1PCa had lower amounts of C (Fig. 3A). For Fe, no differences among inoculation treatments were found in plants grown in RPE (Fig. 3B). Similar to plants grown in PNS, plants grown in RPE and inoculated with *P. putida* M5TSA contained the most Mg (Fig. 3C).

In plants inoculated with *B. megaterium* M1PCa, high levels of N were detected. The lowest level of N occurred in plants inoculated with *A. vinelandii* M2Per, whereas the highest values of N were detected in plants inoculated with the positive control, *B. pumilus* ES4, and grown on the poorest substrate PE (Fig. 3D). For P, plants grown on RPE and inoculated with *B. megaterium* M1PCa had the highest amount of P. The lowest P was found in plants inoculated with *A. vinelandii* M2Per. Higher values occurred in plants grown on RPE, compared to PNS (Fig. 3E). Similarly, Zn was higher in plants inoculated with *B. megaterium* M1PCa and lowest in the positive control *B. pumilus* ES4. The highest values of Zn occurred in plants inoculated with *A. vinelandii* M2Per and non-inoculated plants growing on PNS (Fig. 3F). For Al, high levels occurred in plants inoculated with *B. megaterium* M1PCa, comparable only with those plants grown on PNS and inoculated with *P. putida* M5TSA. The lowest level of Al occurred in plants inoculated with the positive control *B. pumilus* ES4 and grown on PE (Fig. 3G). The highest level of Na occurred in plants inoculated with *B. megaterium* M1PCa and grown on RPE and the lowest level occurred in plants inoculated with *A. vinelandii* M2Per (Fig. 3H). *B. megaterium* M1PCa led to more Ca in plants grown on RPE, which was similar to plants in the PNS. The lowest level of Ca occurred in plants inoculated with the positive control *B. pumillus* ES4 and grown on the poorest substrate PE (Fig. 3I). For Si, there was a marked effect of *A. vinelandii* M2Per in plants grown in RPE; however, the highest value occurred in plants inoculated with *P. putida* M5TSA growing on PNS (Fig. 3J). Lastly, the level of K in RPE was similar among the different treatments

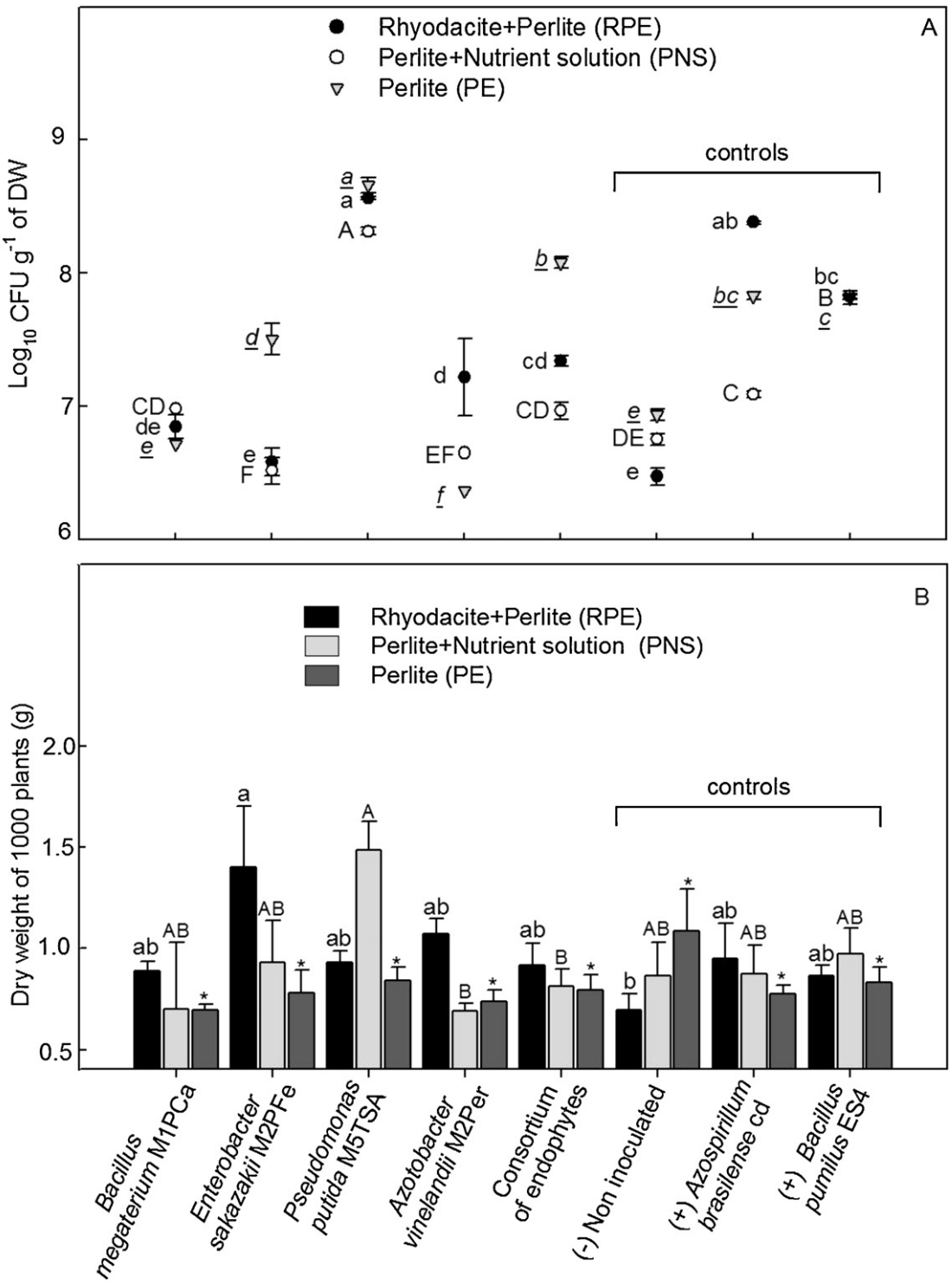

Fig. 2. (A) Populations of recovered endophytic bacteria from inoculated plants and (B) dry biomass of *Mammillaria fraileana* inoculated with endophytes and grown on different substrates. (A) Data was analyzed by one-way ANOVA ($P < 0.001$) and means comparison of $\log_{10}$ CFU g^{-1} per dry weight of plants by the Tukey–Kramer HSD at $P < 0.05$. Comparisons among bacterial inoculations of each substrate are among letters of the same type (compare data denoted with capital, lower case and underlined letters, separately). Bars represent SE. The absence of a bar indicates a negligible SE. CFU in plants that were not inoculated indicates natural populations of endophytic bacteria. (B) Different letters within each substrate indicate significant differences at $P < 0.05$. Bars represent SE. *No significant effect of inoculation on biomass of plants. Controls: (−) not inoculated = negative control, (+) positive control = plant growth-promoting bacteria.

with bacteria, and their level in plants was generally lower than those detected in PNS or PE (Fig. 3K). In general, the effect of the consortium of endophytes and *Enterobacter zakazakii* M2PFe was at intermediate levels among all inoculation treatments (Table 2, Fig. 3). No perceptible signs of toxicity were observed in plants with increased levels of the elements measured.

3.4. Effect of endophytic bacteria on the nitrogen content of plants and nocturnal acid accumulation

Total N in plants grown on RPE varied among inocula ($F_{3,20} = 3.6$, $P < 0.01$, $n = 24$). The effect of two putative PGPB endophytic bacteria isolated from *M. fraileana* differed significantly ($P < 0.05$).

Table 1
Values of two-way ANOVA analyzing the effect of inoculation and substrates on the abundance of elements in *Mammillaria fraileana* cacti.

Element	Relative abundance[a] (%)	*F* values		
		Substrate[b] (d.f. 2, 72)	Inoculum[c] (d.f. 7, 72)	Substrate × inoculum (d.f. 14, 72)
C	45.64 ± 0.54	3.09*	2.4*	1.47 NS
N	40.73 ± 0.37	3.29*	31.87***	14.82***
Zn	4.86 ± 0.18	0.77 NS	12.79***	7.89***
Na	3.02 ± 0.09	10.72***	19.27***	3.59**
Si	2.42 ± 0.18	7.34**	19.13***	4.03***
Ca	1.05 ± 0.04	15.46***	9.54***	7.50***
K	1.05 ± 0.08	38.17***	10.78***	3.02***
Al	0.96 ± 0.04	9.62***	7.14***	4.19***
P	0.42 ± 0.02	1.49 NS	5.48***	3.21***
Mg	0.34 ± 0.02	8.28***	9.16***	1.48 NS
Fe	0.11 ± 0.01	13.24***	2.63*	1.51 NS
Cu	Trace			
Mn	Trace			

Two-way ANOVA after arcsin transformation of relative abundance of elements obtained by X-ray analysis.
[a] Values of relative abundance of elements, non-transformed ± SE.
[b] Substrates: rhyodacite + perlite (RPE); perlite supplemented with Hoagland's nutrient solution (PNS); and perlite alone (PE).
[c] Inocula: 5 treatments with endophytic bacteria isolated from roots of *M. fraileana*, two positive bacterial controls, and non-inoculated negative control.
* $P < 0.05$.
** $P < 0.001$.
*** $P < 0.0001$.
NS = not significant.

Table 2
Means comparisons for the overall effect of inoculation of endophytes on the abundance of 11 elements in plants of *Mammillaria fraileana* grown in three substrates.

Inoculation treatment	Al	C	Ca	Fe	K	Mg	N	Na	P	Si	Zn
Bacillus megaterium M1PCa	ab	c	a	ab	b	ab	bc	a	a	bc	ab
Enterobacter sakazakii M2PFe	bc	ab	ab	b	cd	cd	b	cd	abc	d	cd
Pseudomonas putida M5TSA	a	bc	a	ab	a	a	de	ab	a	a	de
Azotobacter vinelandii M2Per	ab	a	bc	a	bc	d	e	d	c	ab	abc
Consortium of endophytes	bc	ab	bc	ab	bcd	bcd	bc	bc	ab	cd	abc
Controls[a]											
(−) Not inoculated	bc	a	bc	ab	bcd	bcd	cde	cd	abc	cd	a
(+) *Azospirillum brasilense* cd	ab	a	bc	ab	ab	abc	cd	cd	abc	ab	cde
(+) *Bacillus pumilus* ES4	c	ab	c	ab	d	bcd	a	cd	bc	d	e

Substrates: rhyodacite + perlite (RPE); perlite supplemented with Hoagland's nutrient solution (PNS); and perlite alone (PE).
Two-way ANOVA after arcsin transformation of relative abundance of elements and means comparison by Tukey–Kramer HSD ($P < 0.05$). Different lower case letters within each element indicate differences among inoculation treatments.
[a] Controls: (−) not inoculated = negative control, (+) positive control = plant growth-promoting bacteria.

Plants inoculated with *P. putida* M5TSA had the highest content of N, whereas plants inoculated with the nitrogen-fixing strain *A. vinelandii* M2Per and the positive control PGPB (the diazotroph *A. brasilense* Cd.) had lower levels of N, but were not significantly higher than what occurred in plants that were not inoculated (Fig. 4A).

All inoculated plants showed an increase in nocturnal acid, which indicated carbon dioxide uptake by CAM-type photosynthesis. Accumulation of nocturnal acid in plants differed among inoculated bacterial species ($F_{3,20} = 6.27$, $P < 0.004$). Plants inoculated with *P. putida* M5TSA or *A. vinelandii* M2Per contained more acid. Intermediate levels of acid were found in plants inoculated with the positive control *A. brasilense* Cd; the plants that were not inoculated had the lowest increase in acid (Fig. 4B).

4. Discussion

Exploring the role of endophytic bacteria in plants, especially those that are also PGPB, is gaining interest in commercial applications, such as crop production, phytoremediation, reforestation, and medicine (Hung et al., 2007; Puente et al., 2009b; Strobel, 2007). Although wild plants are presumed to harbor unique populations of endophytic PGPB (Hung et al., 2007; Puente et al., 2009a), there are only a handful of studies linking the association of plants and endophytic bacteria in nutrient-poor environments (Dalton et al., 2004; Doty et al., 2009; Puente et al., 2009a,b). We intended to elucidate if several parameters of plant growth were affected by inoculation with native, culturable bacterial endophytes of *M. fraileana*, a pioneer cactus growing on volcanic rock. This study demonstrated that, when taken as a whole, inoculation significantly influenced plant growth by increasing (1) biomass, (2) total nitrogen content, (3), mobilization of elements into the plant, and (4) photosynthetic activity.

4.1. Biomass

Despite the characteristically slow growth of cacti and particularly the genus *Mammillaria* (Rojas-Arechiga and Vazquez-Yanes, 2000), there was a noticeable effect of inoculating plants with endophytic bacteria. Complementary to our findings, Puente et al. (2009a) found that inoculation with other endophytic bacteria enhanced dry weight, volume, root length, and height of seedlings of the giant cardon cactus *P. pringlei* and suggested that these bacteria are essential for development of this plant species in rocky habitats. We found that one endophyte, *P. putida* M5TSA, was related to higher biomass in the substrate supplemented with a nutrient solution, while another endophyte *E. sakazakii* M2PFe was associated with plants growing on rhyodacite, the volcanic rock where *M. fraileana* grows abundantly (Lopez et al., 2009). Hence, it is

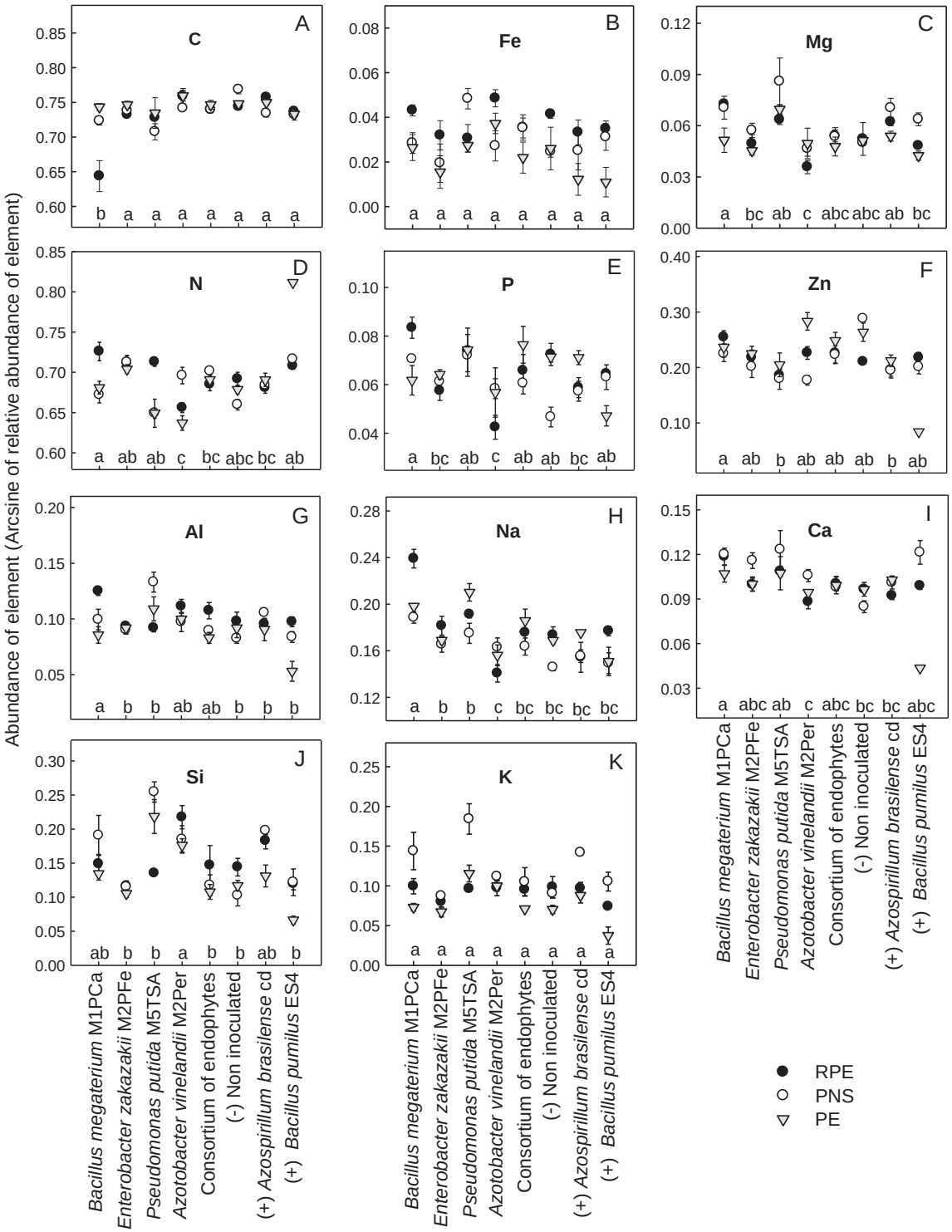

Fig. 3. Effects of inoculation with endophytic bacteria and substrates (three growth substrates) on the abundance of (A) carbon, (B) iron, (C) magnesium, (D) nitrogen, (E) phosphorus, (F) zinc, (G) aluminium, (H) sodium, (I) calcium, (J) silicon, and (K) potassium in the cactus *Mammillaria fraileana*. Substrates: RPE, rhyodacite + perlite; PNS, perlite supplemented with Hoagland's nutrient solution; and PE, perlite alone. Means for RPE with different lower case letter differ significantly at $P < 0.05$ by the Tukey–Kramer HSD test. Bars represent SE, the absence of a bar indicates a negligible SE. Controls: (−) not inoculated = negative control, (+) positive control = plant growth-promoting bacteria.

likely that rocks, particularly their chemical composition, favor participation of some endophytes over others. The marked effect of the *E. sakazakii* strain might explain its traits that are potentially beneficial to plant growth, such as solubilization of phosphate (Lopez et al., 2011; Schmid et al., 2009) and production of siderophores and indole-3-acetic acid in several strains of this species (Schmid et al., 2009).

4.2. Mobilization of elements

M. fraileana responded to inoculation with endophytic bacteria mainly by increasing concentrations of elements. Mobilization of elements from rocks to plants may occur by solubilizing activity of various functional types of bacteria (both rhizoplane and endophytic dwellers) that colonize cactus roots and by organic acids

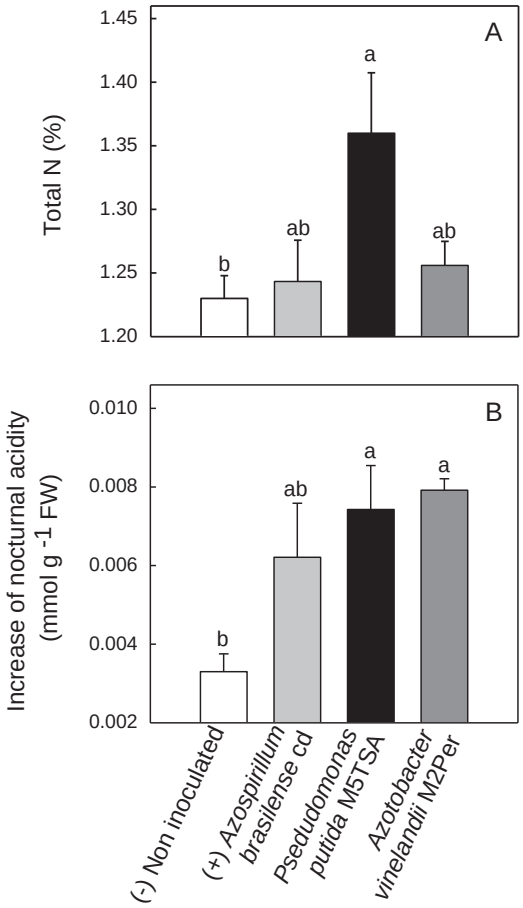

Fig. 4. Effect of inoculation with endophytic bacteria on (A) nitrogen content and (B) photosynthetic activity (increase in nocturnal acidity) in the cactus *Mammillaria fraileana* grown on RPE substrate prepared from ground rhyodacite rocks and perlite. Different lowercase letters, in each subfigure separately, differ significantly at $P < 0.05$ by the Tukey–Kramer HSD test. Bars represent SE. Controls: (−) not inoculated plants = negative control, (+) positive control = plant growth-promoting bacteria.

contained in root exudates (Carrillo et al., 2002; Lynch and Whipps, 1990; Puente et al., 2004a,b, 2009a). Roots and root-associated bacteria induce physicochemical changes in the rhizosphere, which influences mineral uptake and rock weathering (Calvaruso et al., 2006; Hinsinger, 1998). Endophytes may also affect mineral uptake indirectly through chemical signals sent to the roots to produce chemical modifications of the rhizosphere or changes in source-sink dynamics in the plant (Malinowski et al., 2000).

Our data indicated that levels of elements in plants vary across combinations of substrates and inoculants. This strongly indicates that mobilization of elements is not a straightforward and simple phenomenon, but a complex process that depends on chemical composition of rocks, binding strength of elements in the minerals, and relative proportion of elements. However, further analyses of these abiotic parameters in conjunction with the biotic part of plant and bacteria are merited and still pending. Similar results for the endophytic fungi *Neotyphodium coenophialum* was reported by Malinowski et al. (2000), where mineral uptake in tall fescue grass was influenced by availability of phosphorus in the nutrient solution. In our study, high availability of nutrients in the substrate supplemented with nutrient solution seemed to intensify the effect of *P. putida* M5TSA in mobilizing minerals.

In the substrate containing rhyodacite, representative of the indigenous habitat of *M. fraileana*, mobilization was partly influenced by the abundance of elements in the rock minerals, which are rich in Si, Al, Fe, Ca, and Na, but low in P and N (Lopez et al., 2009) and strongly influenced by inoculation of *B. megaterium* M1PCa and *A. vinelandii* M2Per. These two strains have traits for weathering of rhyodacite *in vitro* (Lopez et al., 2011). However, in nature, even when Ca was not abundant in the soil, some cacti of the genus *Cephalocereus* contained calcium oxalate crystals (CaC_2O_4; raphides) that also contain other elements, such as Si, Mg, Na, K, Cl, and Fe (Bárcenas-Argüello et al., 2010). Accumulation of Si, Na, and Al does not appear to have a negative effect on plants because cacti are tolerant of potentially stressful levels of elements that are often toxic to other plants (Nobel, 1998). Our results indicate that participation of certain combinations of endophytes involved in mobilizing elements is determined by the composition of the substrate. Variation in response to mineral status in endophyte-colonized plants may also aid survival of plants under a wide range of environmental conditions (Malinowski et al., 2000).

4.3. Photosynthetic activity and nitrogen content in plants

Among adaptations of cacti to arid environments, crassulacean acid metabolism (CAM) is a physiological adaptation that increases efficiency of water use because the stomata open only at night when CO_2 is fixed and air temperatures are lower, relative humidity is higher, and evaporation is lower (Lüttge, 2004). CO_2 is incorporated into phosphenolpyruvate, leading to formation of four-carbon organic acids that are stored in the vacuoles of chlorenchyma cells (Kluge and Ting, 1978). Accumulation of nocturnal acid, the outcome of CAM, is affected by temperature and light intensity (Hernandez-Gonzalez and Briones, 2007; Nobel and Hartsock, 1983) and plant water status (Dodd et al., 2002). There is no previous mention of changes occurring in this photosynthesis pathway in cacti-microbe interactions. In this study, 6.5-month-old (~5 mm high) plants, whether inoculated or not, showed fluctuation in the content of organic acids with significant increases during the night (dawn measurement) and decreases during the day (dusk measurement) (Lüttge, 2004); hence, inoculation with endophytic bacteria enhanced fixation of CO_2 by the CAM pathway of photosynthesis by an unknown mechanism. Support for the claim of potential effects of PGPB on photosynthesis is that inoculation with the PGPB *A. brasilense* (a putative endophyte, serving as a positive control in this study) enhanced accumulation of all major and auxiliary photoprotective phostosynthetic pigments of wheat plants (Bashan et al., 2006a) and enhanced common photosynthesis (*via* the C_3 pathway) (Tsimilli-Michael et al., 2000).

Photosynthetic capacity is strongly correlated with the amount of nitrogen in photosynthetic organs because a large part of the nitrogen in a plant is invested in the photosynthetic apparatus (Field and Mooney, 1986; Yasumura et al., 2006). In our study, the higher content of nitrogen induced by endophytes, was accompanied by higher photosynthetic activity. The effect of the diazotroph *A. vinelandii* M2Per on nitrogen content suggests the contribution of nitrogen to the plant through nitrogen fixation. High levels of nitrogen fixation by other endophytic diazotrophic bacteria enhanced growth of the giant cardon cactus *P. pringlei* (Puente et al., 2009a,b). The relevance of nitrogen fixation for rock-colonizing plants is considerable because there is no biological nitrogen fixation in rocks without vegetation (Puente et al., 2009b). Therefore, diazotrophic endophytic bacteria play an essential role for pioneer plants on rocky substrates containing practically no organic material (Doty et al., 2009; Puente et al., 2009a,b).

The high nitrogen content in plants inoculated with the endophyte *P. putida* M5TSA might be explained by mechanisms other than nitrogen fixation, since this is not a diazotrophic species (Lopez et al., 2011). Many non-diazotrophic PGPB enhance nitrogen uptake by plant roots (Bashan and de-Bashan, 2005). Nonetheless, the source of nitrogen in *M. fraileana* growing on rhyodacite can only

be guessed because the rocks do not contain bound nitrogen (Lopez et al., 2009). On the other hand, the PGPB, *P. putida*, has the capacity to synthesize siderophores and thereby provide iron for the plant (Caron et al., 1995), can lower growth-inhibiting levels of ethylene in plant tissues (Glick et al., 1998), and can secrete indole-3-acetic acid (Patten and Glick, 2002).

4.4. Detection and populations of endophytic bacteria

All strains that were inoculated were detected by re-isolation and sequencing of the 16S DNA of these strains. These results were supported by parallel experiments that tested re-colonization by *P. putida* M5TSA, *E. sakazakii* M2PFe, and *B. megaterium* M1PCa of roots of *M. fraileana* (Lopez et al., 2011). Plants that were surface-sterilized were inoculated and later analyzed by histological methods by specific detection using immuno-localization of the original inoculum. The three isolates successfully penetrated the plants and became established as endophytes. Re-colonization by the three isolates occurred predominantly at the base of the main root, decreasing toward above-ground tissues and toward root tips (Lopez et al., 2011).

In this study, 3.5 months after inoculation, we recovered endophytes in *M. fraileana* the resemble levels of endophytic populations (3–6 $\log_{10}$ CFU g^{-1} fresh weight) of indigenous and introduced bacteria, where variations are attributed to plant source, plant age, tissue type, capacity of colonization, and the environment (Kobayashi and Palumbo, 2000; Zinniel et al., 2002). Because our previous study detected many unculturable bacteria in seeds and plants (Lopez et al., 2011) and the current study inoculated seedlings only with culturable strains, we suggest that the net effect of inoculation, in terms of growth promotion and mineral mobilization, represents only part of the observed effect. Consistently high populations of bacteria in plants inoculated with *P. putida* M5TSA and grown on different substrates reflect the general ability of *Pseudomonas* sp. to establish high densities in the rhizosphere and colonize plants internally (Elvira-Recuenco and van Vuurde, 2000).

Even though plants that were not inoculated had exhibited culturable endophytic bacteria, their populations were lower, compared to inoculated plants. Detection of *Methylobacterium sp*. in untreated plants was an unexpected finding because: (1) No culturable bacteria were detected in seeds and seedlings before inoculation (Lopez et al., 2011) and (2) Pots with plants were maintained aseptically during the greenhouse experiment. Explanations might be that endophytic populations in seeds and seedlings were either at undetectable levels or in a physiological state that render them unculturable (Hallmann et al., 1997); once the plant developed, the bacteria become culturable. On the other hand, the presence of *Achromobacter xylosoxidans* in inoculated plants might be related to the induction phenomenon of no culturable endophytes by the inoculation with other bacterium (Podolich et al., 2009).

In conclusion, this study provides experimental evidence for improving growth of the cactus *M. fraileana* by mobilizing elements from rock and increasing photosynthetic activity, nitrogen content, and biomass by inoculation with native endophytic bacteria. Taken together with the studies of endophytic PGPB from another cactus, the giant cardon (Puente et al., 2009a,b), it appears that the relation between endophytes and desert plants is more common in deserts than was expected. The objective of this line of research is to show that the interaction of pioneering cacti with their bacterial endophytes may give cacti an ecological advantage on barren rock surfaces and enable cacti to grow in places where plants normally cannot grow. Through colonization and establishment of pioneer plants, and perhaps with help of rhizoplane and endophytic bacteria capable of weathering rocks (Lopez et al., 2011; Puente et al., 2004a, 2009a), soil is created, which then facilitates colonization by other desert plants and contributes to plant diversity in dry regions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2012.02.014.

References

Anderson, E.F., 2001. The Cactus Family. Timber Press, Portland, OR.
Azevedo, J.L., Maccheroni, J.W., Pereira, J.O., Araújo, W.L., 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron. J. Biotechnol. 3, 40–65, http://www.ejb.org/content/vol3/issue1/full/4 (online).
Bárcenas-Argüello, M.L., Gutiérrez-Castorena, M., Terrazas, T., López-Mata, L., 2010. Rock-soil preferences of three Cephalocereus (Cactaceae) species of tropical dry forests. Soil Sci. Soc. Am. J. 74, 1374–1382.
Bashan, Y., Li, C.Y., Lebsky, V.K., Moreno, M., de-Bashan, L.E., 2002. Primary colonization of volcanic rocks by plants in arid Baja California, Mexico. Plant Biol. 4, 392–402.
Bashan, Y., de-Bashan, L.E., 2005. Bacteria/plant growth-promotion. In: Hillel, D. (Ed.), Encyclopedia of Soils in the Environment, vol. 1. Elsevier, Oxford, pp. 103–115.
Bashan, Y., Bustillos, J.J., Leyva, L.A., Hernandez, J.P., Bacilio, M., 2006a. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. Biol. Fertil. Soils 42, 279–285.
Bashan, Y., Vierheilig, H., Salazar, B.G., de-Bashan, L.E., 2006b. Primary colonization and breakdown of igneous rocks by endemic, succulent elephant trees (Pachycormus discolor) of the deserts in Baja California, Mexico. Naturwissenschaften 93, 344–347.
Bashan, Y., Khaosaad, T., Salazar, B.G., Ocampo, J.A., Wiemken, A., Oehl, F., Vierheilig, H., 2007. Mycorrhizal characterization of the boojum tree, *Fouquieria columnaris*, an endemic ancient tree from the Baja California Peninsula, Mexico. Trees Struct. Funct. 21, 329–335.
Benhamou, N., Gagné, S., Quéré, D.L., Dehbi, L., 2000. Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. Biochem. Cell Biol. 90, 45–56.
Calvaruso, C., Turpault, M.P., Frey-Klett, P., 2006. Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. Appl. Environ. Microbiol. 72, 1258–1266.
Caron, M., Patten, C.L., Ghosh, S., Glick, B.R., 1995. Effects of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12–2 on the physiology of canola roots. Plant Growth Regul. 23, 297–302.
Carrillo, A.E., Li, C.Y., Bashan, Y., 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. Naturwissenschaften 89, 428–432.
Chi, F., Shen, S.H., Cheng, H.P., Jing, Y.X., Yanni, Y.G., Dazzo, F.B., 2005. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl. Environ. Microbiol. 71, 7271–7278.
Dalton, D.A., Kramer, S., Azios, N., Fusaro, S., Cahill, E., Kennedy, C., 2004. Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. FEMS Microbiol. Ecol. 49, 469–479.
Danhorn, T., Fuqua, C., 2007. Biofilm formation by plant-associated bacteria. Ann. Rev. Microbiol. 61, 401–422.
Dodd, A.N., Borland, A.M., Haslam, R.P., Griffiths, H., Maxwell, K., 2002. Crassulacean acid metabolism: plastic, fantastic. J. Exp. Bot. 53, 569–580.
Doty, S.L., 2008. Enhancing phytoremediation through use of transgenics and endophytes. New Phytol. 179, 318–333.
Doty, S.L., Oakley, B., Xin, G., Kang, J.W., Singleton, G., Khan, Z., Vajzovic, A., Staley, J.T., 2009. Diazotrophic endophytes of native black cottonwood and willow. Symbiosis 47, 27–33.
Elvira-Recuenco, M., van Vuurde, J.W.L., 2000. Natural incidence of endophytic bacteria in pea cultivars under field conditions. Can. J. Microbiol. 46, 1036–1041.
Fahey, J.W., Dimock, M.B., Tomasino, S.F., Taylor, J.M., Carlson, P.S., 1991. Genetically engineered endophytes as biocontrol agents: a case study in industry. In: Andrews, J.H., Hirano, S.S. (Eds.), Microbial Ecology of Leaves. Springer-Verlag, New York, pp. 402–411.

Field, C., Mooney, H.A., 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish, T.J. (Ed.), On the Economy of Form and Function. Cambridge University Press, Cambridge, pp. 25–55.
Forchetti, G., Masciarelli, O., Izaguirre, M., Alemano, S., Alvarez, D., Abdala, G., 2010. Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. Curr. Microbiol. 61, 485–493.
Germaine, K.J., Liu, X., Garcia-Cabellos, G., Hogan, J.P., Ryan, D., Dowling, D.N., 2006. Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol. Ecol. 57, 302–310.
Glick, B.R., Penrose, D.M., Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor. Biol. 190, 63–68.
Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43, 895–914.
Hallmann, J., Quadt-Hallmann, A., Rodríguez-Kábana, R., Kloepper, J.W., 1998. Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. Soil Biol. Biochem. 30, 925–937.
Hardoim, P.R., van Overbeek, L.S., Van Elsas, J.D., 2008. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 16, 463–471.
Hernandez-Gonzalez, O., Briones, V.O., 2007. Crassulacean acid metabolism photosynthesis in columnar cactus seedlings during ontogeny: the effect of light on nocturnal acidity accumulation and chlorophyll fluorescence. Am. J. Bot. 94, 1344–1351.
Hinsinger, P., 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Adv. Agron. 64, 225–265.
Hung, P.Q., Kumar, S.M., Govindsamy, V., Annapurna, K., 2007. Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. Biol. Fertil. Soils 44, 155–162.
Kloepper, J.W., Ryu, C.M., 2006. Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz, B., Boyle, C., Sieber, T. (Eds.), Microbial Root Endophytes. Springer-Verlag, Heidelberg, pp. 33–52.
Kluge, M., Ting, I.P., 1978. Crassulacean acid metabolism: analysis of ecological adaptation. In: Ecological Studies Series, vol. 30. Springer-Verlag, Berlin.
Kobayashi, D.Y., Palumbo, J.D., 2000. Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon, C.W., White, J.F. (Eds.), Microbial Endophytes. Dekker, New York, pp. 199–236.
Long, H.H., Schmidt, D.D., Baldwin, I.T., 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. PLoS ONE 3, e2702.
Lopez, B.R., Bashan, Y., Bacilio, M., 2009. Rock colonization plants: abundance of the endemic cactus *Mammillaria fraileana* related to rock type in the southern Sonoran Desert. Plant Ecol. 201, 575–588.
Lopez, B.R., Bashan, Y., Bacilio, M., 2011. Endophytic bacteria of *Mammillaria fraileana*, an endemic rock-colonizing cactus of the southern Sonoran Desert. Arch. Microbiol. 193, 527–541.
Lüttge, U., 2004. Ecophysiology of crassulacean acid metabolism (CAM). Ann. Bot. (Lond.) 93, 629–652.
Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant Soil 129, 1–10.
Malinowski, D.P., Alloush, G.A., Belesky, D.P., 2000. Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. Plant Soil 227, 115–126.
Nobel, P.S., Hartsock, T.L., 1983. Relationships between photosynthetically active radiation, nocturnal acid accumulation, and CO_2 uptake for a crassulacean acid metabolism plant, *Opuntia ficus-indica*. Plant Physiol. 71, 71–75.
Nobel, P., Berry, W.L., 1985. Element responses of agaves. Am. J. Bot. 72, 686–694.
Nobel, P.S., 1998. Environmental Biology of Agaves and Cacti. Cambridge University Press, New York, pp. 175–198.
Patten, C.L., Glick, B.R., 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68, 3795–3801.
Podolich, O., Laschevskyy, V., Ovcharenko, L., Kozyrovska, N., Pirttilä, A.M., 2009. *Methylobacterium* sp. resides in unculturable state in potato tissues *in vitro* and becomes culturable after induction by *Pseudomonas fluorescens* IMGB163. J. Appl. Microbiol. 106, 728–737.
Puente, M.E., Bashan, Y., Li, C.Y., Lebsky, V.K., 2004a. Microbial populations and activities in the rhizoplane of rock-weathering desert plants, I. Root colonization and weathering of igneous rocks. Plant Biol. 6, 629–642.
Puente, M.E., Li, C.Y., Bashan, Y., 2004b. Microbial populations and activities in the rhizoplane of rock-weathering desert plants, II. Growth promotion of cactus seedling. Plant Biol. 6, 643–650.
Puente, M.E., Rodriguez-Jaramillo, M.C., Li, C.Y., Bashan, Y., 2006. Image analysis for quantification of bacterial rock weathering. J. Microbiol. Methods 64, 275–286.
Puente, M.E., Li, C.Y., Bashan, Y., 2009a. Rock-degrading endophytic bacteria in cacti. Environ. Exp. Bot. 66, 389–401.
Puente, M.E., Li, C.Y., Bashan, Y., 2009b. Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. Environ. Exp. Bot. 66, 402–408.
Ramesh, R., Joshi, A.A., Ghanekar, M.P., 2009. Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). World J. Microbiol. Biol. 25, 47–55.
Rennie, R.J., 1981. A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. Can. J. Microbiol. 27, 8–14.
Rojas-Arechiga, M., Vazquez-Yanes, C., 2000. Cactus seed germination: a review. J. Arid Environ. 44, 85–104.
Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., Dowling, D.N., 2008. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 278, 1–9.
Schmid, M., Iversen, C., Gontia, I., Stephan, R., Hofmann, A., Hartmann, A., Jha, B., Eberl, L., Riedel, K., Lehner, A., 2009. Evidence for a plant-associated natural habitat for *Cronobacter* spp. Res. Microbiol. 160, 608–614.
Schulz, B., Boyle, C., 2006. What are endophytes? In: Schulz, B., Boyle, C., Sieber, T. (Eds.), Microbial Root Endophytes. Springer-Verlag, Heidelberg, pp. 1–11.
Singh, B.K., Millard, P., Whitely, A.S., Murrell, J.C., 2004. Unravelling rhizosphere–microbial interactions: opportunities and limitations. Trends Microbiol. 12, 386–393.
Strobel, G., 2007. Plant-associated microorganisms (Endophytes) as a new source of bioactive natural products. In: Kayser, O., Quax, W.J. (Eds.), Medicinal Plant Biotechnology, from Basic Research to Industrial Applications, vol. 1. Wiley-VCH Verlag, Heidelberg, pp. 49–72.
Sturz, A.V., Christie, B.R., Matheson, B.G., Nowak, J., 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol. Fertil. Soils 25, 3–19.
Sturz, A.V., Nowak, J., 2000. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl. Soil Ecol. 15, 183–190.
Tsimilli-Michael, M., Eggenberg, P., Biro, B., Koves-Pechy, K., Voros, I., Strasser, R.J., 2000. Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the polyphasic chlorophyll a fluorescence transient O-J-I-P. Appl. Soil Ecol. 15, 169–182.
van Dam, N.M., Heil, M., 2011. Multitrophic interactions below and above ground: en route to the next level. J. Ecol. 99, 77–88.
Valverde, T., Quijas, S., Lopez-Villavicencio, M., Castillo, S., 2004. Population dynamics of *Mammillaria magnimamma* Haworth (Cactaceae) in a lava-field in central Mexico. Plant Ecol. 170, 167–184.
Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571–586.
Wiggins, I.L., 1980. Flora of Baja California. Stanford University Press, Stanford, CA.
Yasumura, Y., Hikosaka, K., Hirose, T., 2006. Seasonal changes in photosynthesis, nitrogen content and nitrogen partitioning in *Lindera umbellata* leaves grown in high or low irradiance. Tree Physiol. 26, 1315–1323.
Zinniel, D.K., Lambrecht, P., Harris, N.B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C.A., Arunakumari, A., Barletta, R.G., Vidaver, A.K., 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl. Environ. Microbiol. 68, 2198–2208.
Zotz, G., Andrade, J.L., 1998. Water relations of two co-occurring epiphytic bromeliads. J. Plant Physiol. 152, 545–554.