

## **ROCK WEATHERING, PLANT GROWTH-PROMOTING BACTERIA FROM DESERT PLANTS ALLOW THE GROWTH OF CACTUS SEEDLING IN ROCKS**

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### **ABSTRACT**

Light, fluorescent, and field emission scanning electron microscopy, combined with microbial counts on culture media, revealed the presence of numerous microorganisms (bacteria, fungi, and actinomycetes) living as rhizoplane populations of roots of at least three species of cactus and a wild fig tree growing in rocks without soil. These plants are responsible for weathering of volcanic rock in rocky areas of Baja California Sur, Mexico. The dominant bacterial groups colonizing the rhizoplane were fluorescent pseudomonads and bacilli. N<sub>2</sub>-fixation, solubilization of insoluble phosphate, and rock disintegration to a powder by some of the root-colonizing microorganisms was significant. These bacteria were thermotolerant and halotolerant and capable of significantly depleting the useful minerals, such as P, K, Mg, Mn, Fe, Cu, and Zn in the rocks. Cacti inoculated with these bacteria and growing in crushed volcanic rocks grew normally without N and P fertilization for at least 12 months. Uninoculated cacti barely grew and many died. The microbial community survived in the rhizoplane of cacti during the 10-month annual dry season. This study may show the possible involvement of rhizoplane bacteria, possibly plant growth-promoting bacteria, in rock weathering by cacti growing in hot deserts.

### **INTRODUCTION**

Weathering (breakdown) of stones and rocks is caused by physical (weather), chemical (air pollution and acid rain), and biological processes (Goudie and Parker, 1999). Microorganisms found on rock surfaces, in cracks, and within the pore space of sandstone or granite contribute to the breakdown of rocks. Microbial rock weathering is common in all climate zones, is usually very slow, and has been observed in hot and cold deserts. Little is known about microbial weathering mechanisms, except that some microorganisms are acid-producers in culture (Hirsch et al., 1995). Rocks can be solubilized by microorganisms that produce acids as byproducts of their metabolism. This mechanism happens to be beneficial by making elements available to microbes and plants. Some examples of these microbes include bacteria that solubilize otherwise insoluble Fe<sup>+3</sup> from rocks (Adams et al., 1992), phosphate-solubilizers abundant in agricultural fields (Chabot et al, 1996), and solubilization of rocks (Chang and Li, 1998).

Biological weathering of rock minerals by roots and microorganisms plays an indispensable role in maintaining a supply of inorganic nutrients for the plants (Chang and Li, 1998, Hinsinger et al., 1992). The effect of interactions between plants and rhizosphere bacteria in rock weathering and soil formation has been rarely studied, and is mostly

speculative (Berthelin et al., 1991). We previously described several species of desert plants (mainly cacti) growing in the absence of soil on cliffs, rocks, and ancient volcanic flows in hot desert areas in Baja California, Mexico (Bashan et al., 2002). These plants noticeably weather volcanic rocks and cliffs. Our purpose was to identify and observe microbial species residing in the rhizoplane of desert plants and to record their activities and relation with the plants, as it is hypothesized that these microorganisms participate in rock weathering and assist plant growth by supplying nitrogen, soluble phosphorus, and other essential minerals.

## **MATERIALS AND METHODS**

### **General Techniques**

The source of the plants and sampling techniques were described previously in detail (Bashan et al., 2002). Mineral analyses of crushed volcanic rocks were performed by standard atomic absorption techniques and organic acid analysis by gas chromatography. Rock-solubilizing bacteria and phosphate-solubilizing bacteria were isolated and counted, as described earlier (Chang and Li, 1998). Fluorescent pseudomonads from the root surface were enumerated in King's B medium. Bacterial species were identified by standard 16S rRNA analysis. N<sub>2</sub>-fixation of isolates was performed by the acetylene reduction assay. Scanning electron and light microscopy was performed, as described in Bashan et al. (2002). Vital staining of microorganisms on and within roots by fluorescein diacetate was performed according to Söderström (1977). Statistical analysis was performed by one-way (ANOVA) at  $P \leq 0.05$ , using Statistica software (Statsoft, Tulsa, OK).

### **Inoculation of Cardon Cacti Growing in Pulverized Rock with Rhizoplane Isolates**

Volcanic rocks were submerged in 1 N HCl solution overnight at room temperature (28-33°C) to eliminate organic matter, then rinsed several times with deionized water, and dried at 160°C for 2 h. These rocks were pulverized in a mill (Sprecher and Schun Industrial Control) and sieved to 120- $\mu$ m size. Four g were mixed with 23 g of perlite and placed in small black pots. Cardon seeds (*Pachycereus pringlei* (S. Watson) Britton & Rose 1909), the main plant species growing in rocks, were collected from wild cacti located at La Purisima, Baja California Sur, Mexico (Bashan et al., 2002). Seeds were thoroughly washed with 2% detergent (Tween 20) for 10 min to remove residual dust. Their surface was disinfected with 3% NaOCl for 5 min and then rinsed continuously for 10 min with distilled sterile water. Seeds were inoculated by the standard vacuum infiltration technique for this plant species (Puente and Bashan, 1993) with each of the following bacterial species: *Bacillus chitinolyticus*, *B. subtilis* var. 2, *Bacillus pumilus* var. 2, *Citrobacter* sp, where the PGPB *Pseudomonas putida* R-20 (R.M. Osburn, University of California, Berkeley) and *Azospirillum brasilense* Cd (ATCC 29710) served as positive controls. Bacterial isolates were grown in nutrient broth at 30°C for 18 h, harvested by centrifugation at 1000 g for 20 min, and suspended in 0.85 % saline solution to a final concentration of 10<sup>6</sup> cfu/ mL. Ten seeds were placed on the surface of the substrate, previously irrigated with 50 mL distilled water, and covered with a 5 mm layer of the substrate. The study pots were incubated in a growth chamber (Biotronette Mark III) at 30°C under 70  $\mu$ E/m<sup>2</sup>/s light intensity for 12 months. Each pot was irrigated every 15 days with 25 mL of trace elements of Hoagland's nutrient solution without P and N. Plants inoculated with *P. putida* (grown in perlite alone)

were irrigated with Hoagland's nutrient solution containing N and those inoculated with *A. brasilense* (grown in perlite alone) were irrigated with trace elements of Hoagland's nutrient solution without N but with P. Uninoculated plants served as control. One control treatment was irrigated with trace elements of Hoagland's nutrient solution without P and N, and the other positive control treatment was irrigated with full Hoagland's nutrient solution. Substrates, before and after the experiment, were analyzed for their mineral content. At the end of the experiment, plants were extracted, and height, volume, root length, dry weight (from drying oven, 60°C, 120 h), and N content were evaluated. Inoculation of plants was done in 10 replicates, where each pot, containing 3 seedlings after thinning, served as a replicate.

## RESULTS AND DISCUSSION

In the arid Baja California Peninsula, desert plants (mainly cacti) are well adapted to water scarcity and harsh climatic conditions. In desert highlands, cacti seedlings grow out of rocks and cliffs (Bashan et al., 2002).

All cacti roots were colonized by microbes, verified by three independent microscopic techniques. However, roots were not evenly colonized; young roots were heavily colonized, while older roots lacked a detectable population of microbes. Roots were colonized in the long dry season (10 months) and in the short "wet" season (2 months). Microbial colonization of these plant roots, growing in the absence of measurable amounts of soil ("aeroponic"-like growth) was dense and the type of microbes present was dependent on the season. Specifically, during the dry season, the main colonizers were fungi. Bacteria, mainly having dense fibrous material (pili) were also present, usually close to the fungal hyphae. During the wet season, colonizers of the three cactus species were bacteria, and to a lesser extent, fungi. Vital staining proved that all were alive.

Cellular and colonial bacterial morphotypes among the species were many and varied. In general, colonization of the root surface resembled that of the plant growth-promoting bacterium *Azospirillum* sp. on other plant species; abundant fibrous material anchored the microbes to the root surface and haloes were produced in the mucigel layer covering the roots. The microorganisms persisting in the roots of cacti growing in these rocks are presumably drought resistant and thermotolerant, surviving the long summer at elevated temperatures (over 60°C). The mechanism for this survival is unknown, since few bacterial spores were detected. A large proportion of the cultured bacteria were fluorescent pseudomonads. Seven species of rhizoplane bacteria that, in initial screening showed capacity to weather rocks, were molecularly identified as *Bacillus chitinolyticus*, *B. subtilis* var. 2, *B. fusiformis*, *B. pumilus*, *B. pumilus* var. 2, *Actinomadura oligospora*, and *Citrobacter* sp.

Four of the identified bacteria, together with two PGPB species capable of rock weathering serving as positive controls (*A. brasilense* Cd and *Pseudomonas putida*), were evaluated for rock weathering capacity. Significant reduction in the quantity of the nine elements tested was observed by all tested bacteria, albeit to different degrees, depending on the bacterial species. Maximum reductions observed were: K<sub>2</sub>O (up to 79.8%), Ca<sup>+2</sup> (up to 45.8%), Na (up to 89.2%), Fe<sub>2</sub>O<sub>3</sub> (up to 30.5%), Cu<sup>+2</sup> (up to 49.1%) and P<sub>2</sub>O<sub>5</sub> (up to 31.2%) (Fig 1).

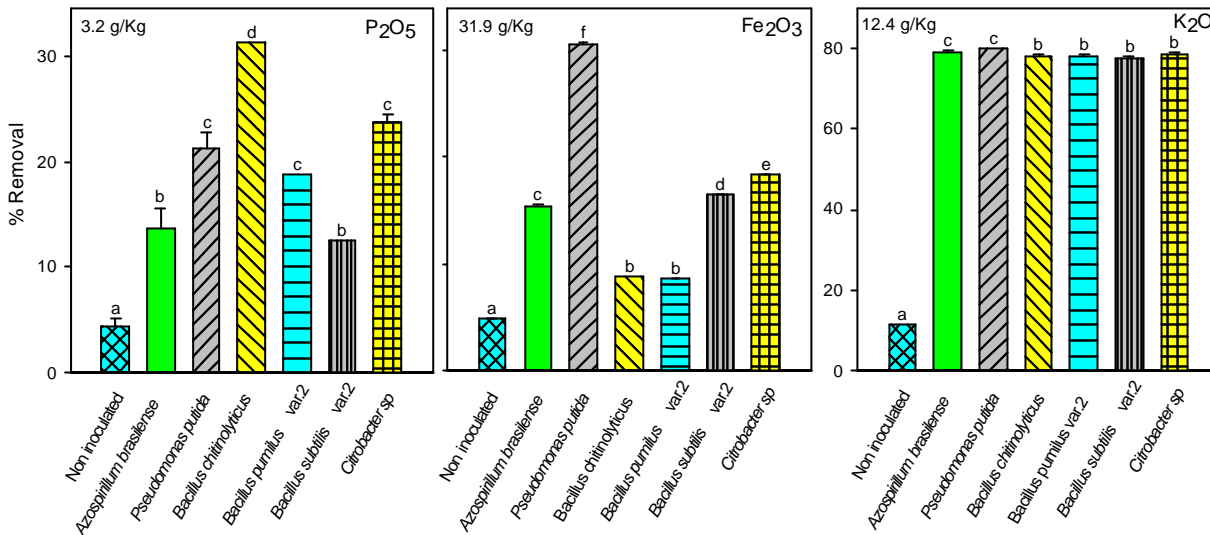


Fig. 1. Removal of P<sub>2</sub>O<sub>5</sub>, Fe<sub>2</sub>O<sub>3</sub>, and K<sub>2</sub>O from pulverized volcanic rocks in vitro by four cactus rhizoplane bacteria after 28 days of incubation. The PGPBs, *P. putida* and *A. brasilense*, served as positive controls. Numbers in each subfigure indicate the quantity of the mineral in the rock. Columns in each subfigure denoted by a different letter differ significantly at  $P \leq 0.05$  in one-way ANOVA. Bars represent SE. Absence of bars means that the SE is minimal.

In vitro, 18 strains were evaluated for their ability to dissolve 3 sources of insoluble phosphate on two solid media. None of the strains had clear capacity to dissolve AlPO<sub>4</sub>. Three strains had a limited ability to dissolve FePO<sub>4</sub> · 2H<sub>2</sub>O, and most strains were capable of dissolving Ca<sub>10</sub>(OH)<sub>2</sub>(PO<sub>4</sub>)<sub>6</sub>. Also in vitro, 7 strains were capable of dissolving pulverized marble and limestone to a great extent.

In winter, average air temperature at 20 cm above the ground was 31°C and rock cavity temperature was 40°C. In the hottest month, the air temperature was over 42°C (almost no shade on the volcanic rock surface) and the rock cavity temperature was over 60°C. All identified bacteria were tested for their ability to withstand high temperatures when growing in liquid culture. None of them grew at 55°C, yet all grew well at 45 and 50°C. NaCl tolerance to 3% (one of the main weathered minerals) was determined for all the bacteria grown in nutrient agar and tryptic soy agar. Eighteen rhizoplane strains isolated from three desert plants (*P. pringlei* and *Opuntia cholla* cacti and wild fig tree *Ficus palmeri*) growing at two locations, showed significant in vitro N<sub>2</sub>-fixation ability (acetylene reduction assay) within the range of 71-180 nmole ethylene/h. All these strains produced several organic acids in significant quantities in liquid culture supplemented with pulverized volcanic rocks and other rocks (data not shown).

Several strains (both N<sub>2</sub>-fixers and P-solubilizers) were individually inoculated onto seeds of the giant cardon cactus. After 12 months, where plants were irrigated only with micronutrients solution lacking N and P, significant growth promotion was observed, including increase in dry weight, volume (Fig 2), height of plants, and size of main root (data not shown). Uninoculated control plants died or were significantly smaller. These results suggest that these microorganisms may serve as plant growth-promoting bacteria for arid land plants used for re-vegetating deserts. Previously, these desert plants responded

positively to inoculation to agricultural PGPBs (Puente and Bashan 1993, Bashan et al., 1999; Carrillo et al., 2002).

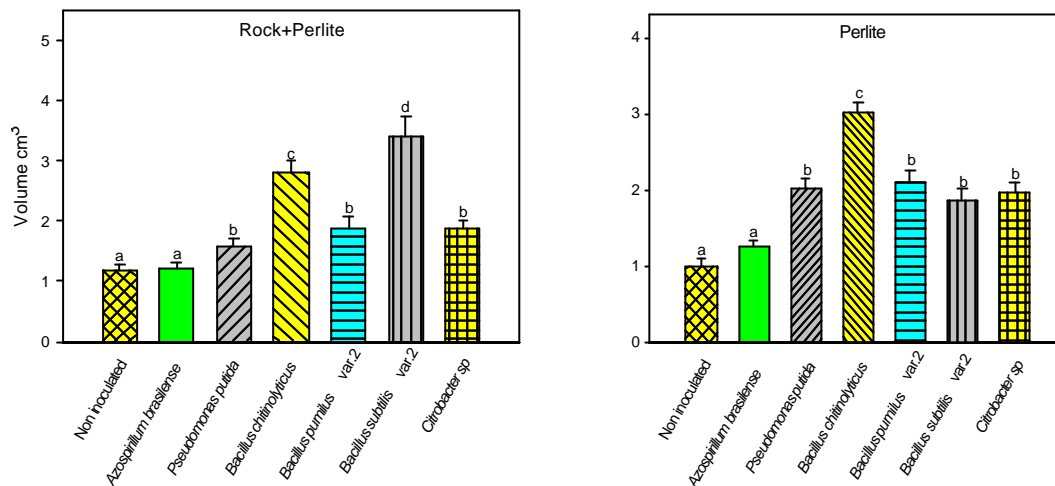


Fig. 2. Growth promotion of cactus seedlings, expressed as volume of plants, after 12 months incubation with four cactus rhizoplane bacteria growing in perlite and in pulverized volcanic rocks with perlite. The PGPBs, *P. putida* and *A. brasilense*, served as positive controls. Columns in each subfigure denoted by a different letter differ significantly at  $P \leq 0.05$  in one-way ANOVA. Bars represent SE.

In summary, this study points out that cacti roots growing in rocks, in the absence of soil, have a massive population of rhizoplane microorganisms comparable to rhizosphere populations on roots of crop plants. These microbes, together with the roots they colonize, may be a significant weathering factor of volcanic rocks in hot deserts.

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