ELSEVIER

Research article

Activity of two catabolic enzymes of the phosphogluconate pathway in mesquite roots inoculated with *Azospirillum brasilense* Cd

Luis A. Leyva [a], Yoav Bashan [a,b,*]

[a] *Environmental Microbiology Group, Northwestern Center for Biological Research (CIBNOR), Mar Bermejo 195, Colonia Playa Palo de Santa Rita, La Paz, BCS 23090, Mexico*
[b] *Department of Soil, Water and Environmental Science, 429 Shantz Building #38, 1200 E. South Campus Dr., The University of Arizona, Tucson, AZ 85721-0038, USA*

Abstract

The mesquite amargo (*Prosopis articulate*), one of the main nurse trees of the Sonoran Desert in Mexico, is responsible for major, natural re-vegetation processes. It exudes gluconic acid in root exudates, a favorite carbon source for the plant growth-promoting bacterium *Azospirillum brasilense*. Two enzymes, gluconokinase (EC 2.7.1.12) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44), participating in the phosphogluconate pathway, are active in the bacteria. Bacterial 6-phosphogluconate dehydrogenase is a constitutive enzyme, while gluconokinase is induced upon exposure to gluconic acid. Both enzymes are active in young, non-inoculated mesquite seedlings growing under hydroponic conditions. When *A. brasilense* Cd bacteria are inoculated on the root system, the roots exhibit much higher activity of gluconokinase, but not 6-phosphogluconate dehydrogenase. Mesquite roots exhibit high levels of root colonization by the inoculating bacteria. At the same time, and also for plants growing under sand culture conditions, the seedlings grew taller, greener, had longer leaves, and were heavier.
© 2008 Elsevier Masson SAS. All rights reserved.

Keywords: Azospirillum brasilense; Gluconic acid; Gluconokinase; 6-Phosphogluconate dehydrogenase; Mesquite; Plant growth-promoting bacteria; Plant–bacteria interaction

1. Introduction

The mesquite is one of the most important exploited trees of many deserts worldwide [1]. In northwestern Mexico, very large numbers of mesquite are harvested, legally and illegally, for the charcoal industry and as material for construction in rural areas [2]. In desert and thorn scrublands, mesquite amargo is a major nurse tree. Below its canopy, a "resource soil island" is created [3]. This area is more fertile, contains more organic matter and clay, and contains more available water. Here, many desert plants, including long-lived cacti, grow more prolifically during their initial years [3–6]. It also harbors more soil-dwelling animals, is a nesting location for rodents, and is harvested by farmers and gardeners as a soil amendment. Mesquite, as a legume, is normally colonized by two types of rhizobia [7,8] and by arbuscular mycorrhizae (AM fungi) [9], which significantly contributes to nitrogen and carbon accumulation in soils of arid lands [10] and to the general ecological well-being of deserts [2].

Azospirillum spp. are non-specific plant growth-promoting bacteria (PGPB) that are capable of promoting growth of numerous crop plants [11], wild plants [12], and even unicellular microalgae [13]. It successfully promotes growth of the giant cardon cactus, the landmark species of the Mexican Sonoran Desert [14], as well as other cacti species [15] that co-habitat the Sonoran Desert with mesquite. Inoculation of propagated mesquite cuttings with several PGPB, including *Azospirillum* spp., indicated that inoculation might be beneficial for root

---

* Corresponding author. Environmental Microbiology Group, Northwestern Center for Biological Research (CIBNOR), Mar Bermejo 195, Colonia Playa Palo de Santa Rita, La Paz, BCS 23090, Mexico. Tel.: +52 612 123 8484x 3668; fax: +52 612 125 4710.

E-mail addresses: bashan@cibnor.mx, bashan@cals.arizona.edu (Y. Bashan).

production in the cuttings, but no conclusive evidence was presented [16]. Additional reasons for choosing *Azospirillum* sp. for inoculation of mesquite are that both prefer high temperatures for growth (>30 °C), the technological aspects of *Azospirillum* and mesquite cultivation have been known for decades and can be easily applied; species of *Azospirillum* have a superb ability to colonize roots of most plant species tested and they promote the growth of numerous plant species regardless of whether the bacteria are part of their natural microflora [11]. Apart from nitrogenase and enzymes related to the nitrogen cycle, only a limited number of enzymes related to *Azospirillum*–plant interactions were evaluated [11], mostly related to phytohormone production and regulation *in vitro* [17], but none were directly related to the degradation of gluconate.

Under controlled conditions, most strains of *A. brasilense* use organic acids, such as malic, succinic, α-ketoglutaric, gluconic, or lactic acids [18–20] as their preferred carbon source. Additionally, gluconic acid is one of the favored carbon sources of *A. lipoferum* for producing siderophores [21]. With glucose induction, *A. brasilense* is even capable of producing gluconate [22]. Gluconate was detected in root exudates of *Lotus japonicus* [23], but was not evaluated for mesquite. Finally, gluconate is known to enhance survival of the soil bacterium *Rhodococcus opacus* under water stress [24] and gluconate metabolism was detected in *Klebsiella pneumoniae* during *in vitro* studies [25].

This study attempted to determine if: (1) gluconate is a root exudate of mesquite *P. articulata*; (2) the presence of gluconate induced the activity of two catabolic enzymes in its pathway in *A. brasilense* Cd, working in tandem; (3) inoculation of mesquite roots enhanced these enzymatic activities in the plant; and (4) inoculation with *A. brasilense* positively affected growth of mesquite.

2. Methods

2.1. Organisms

Azospirillum brasilense Cd (DSM 1843) was used as the inoculant. The bacteria were maintained, routinely cultivated, and checked for purity by the standard methods for this species [26]. Mesquite seeds (*Prosopis articulata* S. Watson) were collected from ten native trees (200 g plant^{-1}) located in fields surrounding the settlements of El Centenario and El Comitán, 15 km from La Paz, BCS, Mexico (24°07'36''N, 110°25'48''W) in July–August 2004 and 2005 when seeds of this species mature.

2.2. Seed extraction

Only seeds that dried on the tree inside the pods were used because only these seeds achieved >80% germination. Pods visibly infected (perforated) with beetle larvae of *Acanthoscelides obtectus* (Say) were discarded. Seeds were extracted from the pods by a mechanical mill (perforation foil, 1-cm diameter, P. Felker, personal communication). Pod debris was discarded,

and seeds were again checked for beetle larvae (visible as a black dot on the upper third of the seeds). Healthy seeds were stored at 2–6 °C for several months.

2.3. Hydroponic growth condition of plants

2.3.1. Germination

Seeds were germinated in sealed plastic trays (25 × 40 cm). The trays were disinfected with 3% sodium hypochlorite solution and then rinsed with sterile distilled water. The seeds were placed on sterile paper towels (wetted with distilled water and autoclaved). Seeds were disinfected under low agitation (50 rpm) with the same disinfectant, decanted, and washed five times with sterile distilled water, 1 min per washing. To promote germination, seeds were placed in a steel strainer and immersed in boiling water for 1 min; water was decanted, and seeds were washed with distilled sterile water at room temperature (26–28 °C), and incubated in a container of distilled sterile water at 26–28 °C for 4 h. Seeds were spread on paper towels, about 1 cm apart and incubated in the dark at 33 °C in a growth chamber (Conviron, Model 125L, Manitoba, Canada). After 3 days, the seedlings were placed in a deep tray (25 × 25 × 5 cm) containing 50% (w/v) modified Hoagland's solution [27], incubated for an additional 2–3 days (post-germination) under light (200–220 μmol m^{-2} s^{-1}, photoperiod of 12:12 h) and 70% relative humidity in the same growth chamber. All transfers were performed under a laminar flow hood to minimize environmental contamination (described below). All hydroponic cultures were axenic from the germination phase of seeds to harvesting of plantlets.

2.3.2. Hydroponic growth

After germination, seedlings having a manageable size (∼10-cm-long roots, 5-cm-long cotyledons) were transferred to 30-ml glass tubes (20 mm diameter, screw cap with a small central perforation) containing 25 ml full-strength Hoagland's solution. The roots of the germinating seedling were inserted through the perforation into the nutrient solution. The seedlings were grown under these conditions for an additional 2 days, until the appearance of first true leaf. This was the time for inoculation. Then the seedlings were transferred to a specific photoperiod regime (12 h light, 200 μmol m^{-2} s^{-1}, 33 ± 1 °C in a growth chamber (Conviron). To avoid fungal growth during this period, the roots of the seedlings were treated twice with a fungicide, propyl 3-(dimethylamino) propyl carbamate at 72.2% w/v (Previcur$^{®}$, Bayer, Mexico) in water at 1 and 2 ml l^{-1} water for 30 min at room temperature (26–28 °C), then thoroughly washed with sterile, distilled water and returned to the hydroponics apparatus. To avoid proliferation of microalgae, the hydroponics unit was sealed against light with commercial aluminum foil, leaving only the foliage exposed to light.

2.4. Cultivation of bacteria and inoculation of seedlings

Bacteria were cultivated in a rich tryptone–yeast extract– glucose salts (TYG) medium for 16 h, harvested by centrifugation, washed, and prepared for inoculation in 0.85% NaCl

[28]. Gluconate was added only to *in vitro* experiments (described later). Bacteria were added to the hydroponics unit at a final concentration of 2.5×10^8 CFU ml^{-1}. Plants were incubated for 3 weeks under the conditions described above. During incubation, about 10 ml week^{-1} full-strength Hoagland's nutrient solution was added to compensate for loss of liquid in the hydroponics. If more liquid was needed, sterile, distilled water was added to maintain constant volume. The concentration of *A. brasilense* Cd colonization of roots and identification of the strain was done 15 and 24 days after inoculation by the plate count method on TYG medium using its typical pink colony color as a marker (the only *Azospirillum* strain exhibiting this color) and expressed as CFU g (dw)$^{-1}$ [28].

2.5. Sand substrate cultures

Raising velvet mesquite in sand substrate was performed to evaluate plant growth parameters. Seeds were germinated and inoculated similar to hydroponic cultures. Plantlets were sown in black PVC tubes (30×5 cm) filled with 350 g acid-treated fine river sand (0.1 N HCl for 24 h, then washed several times with distilled water). Pots were fertilized once with 10 ml of Hoagland's solution and incubated under natural illumination at 32 ± 2 °C for 40 days. This experiment was not axenic.

2.6. Evaluation of plant growth parameters

In hydroponic cultures, the total length of the developing foliage and dry weight of seedlings were measured on days 15 and 23 after inoculation, as described elsewhere [29]. In sand substrate cultures, the following plant growth parameters were measured after 25 and 40 days: height, number of leaves, and length of leaves. Total chlorophyll (*a* and *b*) was measured spectrophotometrically at 650 and 665 nm [30]. After 40 days, the plants were removed. Dry weight of leaves and roots was determined separately.

2.7. Quantification of gluconic acid in root exudates by gas chromatography–mass spectroscopy (GC-MS)

After removing the seedlings, 250 ml of substrate solution was dried at 40 °C for 48 h. The dry deposit was derivatized using concentrated, cold (-20 °C) methanol–HCl (1:1 v/v). The mixture was placed in Teflon-caped tubes and heated at 90 °C for 2 h and then cooled to 25 °C. To this solution, 1.5 ml of HPLC-grade hexane was added and gently mixed. The mixture was left to settle for several minutes. The upper phase, containing methyl gluconate, was analyzed by GC-MS (Model GCD Plus, Hewlett Packard, Palo Alto, CA) with a fused silica capillary column (30 m $\times 0.25$ μm inner diameter, Supelco Omegawax, Bellefonte, PA) filled with polyethylene glycol (Sigma–Aldrich) as the stationary phase and helium as the carrier gas. Separation was done according to the manufacturer's instructions: helium flow at 1.08 ml min^{-1} and injector temperature at 250 °C. After injection of the sample, the temperature of the column was subjected to the following sequence: 70 °C for 5 min, increased to 140 °C at a rate of 8 °C min^{-1}. Total

separation time for each sample was 32.5 min at a detector temperature of 260 °C. The quantity of gluconic acid was calculated automatically by the GC-MS, using analytical gluconic acid (Sigma, St. Louis, MO) as the standard. All chemicals used in this study were of analytical grade from Sigma, unless otherwise indicated.

2.8. Enzyme extraction

2.8.1. Bacteria

A 400-ml culture of *A. brasilense* Cd, cultured for 16 h, was centrifuged at $4000 \times g$ for 10 min. The pellet was washed twice in a saline solution (0.85% NaCl), re-suspended in 8 ml "breaking buffer" (BB = 0.01 M Tris [2-amino-(hydroximetyl)-1,3 propanodiol]) at pH 7.2 with 0.01 M MgCl$_2$, 0.001 M dithiothreitol (DDT), as described by Fraenkel and Levisohn [31]. Cells were sonicated (three 30-s cycles and two 60-s cycles, with a setting interval of 1 min on ice between each cycle (Ultrasonic Homogenizer 4710 Series, Cole–Parmer Instruments, Vernon Hills, IL)). Less successful sonication regimes that were tested are not shown. Cell debris was removed by centrifugation at $10,500 \times g$ for 30 min at 4 °C and the supernatant served as the enzyme source for analyses. Only freshly prepared extracts were used.

2.8.2. Roots

Seedlings (five samples per treatment, each containing three seedlings) growing in an axenic hydroponics unit were used. Roots were excised, cut into small pieces (5 mm long), placed in cold BB buffer, and macerated with glass beads using a glass rod in an ice bath. This initial macerate was further treated, and separated, as described for bacteria. Total protein content of each enzymatic source was analyzed by the Commassie Brilliant Blue method [32].

2.9. Enzymatic activities

Analyses were essentially performed as described by Wang and Dykhuizen [33] with small adjustments. To determine gluconokinase (EC 2.7.1.12) activity, the reaction mixture contained 1 ml of 50 mM HEPES buffer, otherwise known as 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-Nasalt at pH 7.6 (JT Baker, Phillipsburg, NJ), 10 mM MgCl$_2$, 3 mM ATP, 1 mM gluconate, 0.2 mM NADP, and one unit of 6-phosphogluconate dehydrogenase, all previously equilibrated at 37 °C and 100 μl enzyme source. Starting the moment the enzyme was added, the enzymatic activity was continuously measured for 10 min at 340 nm and 37 °C by an eight-cell spectrophotometer (UV/VIS Lambda Bio 20, Perkin–Elmer). Two controls were used: a positive, which was 6-phosphogluconate dehydrogenase (Sigma) at 0.2 units of activity (other concentrations are not shown) and a negative, which is a reaction mixture without an enzyme where the volume of the enzymatic extract was replaced by distilled water.

To determine 6-phosphogluconate dehydrogenase (EC 1.1.1.44), a similar procedure as in the previous enzyme analysis was used, but with a different set of reagents: 50 mM

HEPES buffer-Na-salt at pH 7.6, 10 mM MgCl$_2$, 500 μM phosphogluconate, and 0.2 mM NADP. These two enzymes work sequentially; the product of the first (6-phosphogluconate) is the substrate for the second. Therefore, the same commercial enzyme, which was used as a positive control, can serve for both enzymes.

To evaluate the effect of gluconate in the culture medium on enzymatic activity in *A. brasilense* Cd, six concentrations (1, 3, 5, 10, 15, and 20 g l^{-1}) were added to the TYG medium using controls containing no gluconate in the medium. Bacterial growth and enzymatic activity were evaluated as described above. Enzymatic activity for both enzymes was defined as one enzyme unit equals Δabsorbance min^{-1} μg protein^{-1}.

2.10. Experimental design and statistical analysis

Using a randomized block design, each experiment was carried out in triplicate. Each block contained 40 inoculated plants and 40 plants that were not inoculated. Evaluation of bacterial colonization was done in triplicates on 27 plants and plant response to inoculation used 40 pots. After germination, identically sized seedlings (cotyledons about 5 cm long) were used for inoculation. Plants were sampled for testing growth promotion and root colonization by bacteria on day 15 and 24 after inoculation in hydroponics cultures and after 26 and 40 days in sand substrate cultures. Each experiment, colonization or promotion of growth, was repeated twice and the results are the average of the two experiments, i.e., replicates were compared for each independent experiment and the data were pooled for analysis. All data were first analyzed by ANOVA and then by LSD posthoc analysis or by Student's *t*-test at $P \leq 0.05$ with Statistica™ ver. 6.0 software (Kernel release, StatSoft, Tulsa, OK).

3. Results

3.1. Detection and quantification of gluconic acids from root exudates of mesquite seedlings

Using analytical grade gluconic acid as a marker, we detected an average of 20 μg ml^{-1} gluconic acid in root exudates of mesquite seedlings growing in hydroponics units equivalent to about 0.1 mM (retention time of 14.24 min and relation mass/charge of 43). Gluconic acid was the major root exudate detected with the extraction and assessment measure applied. The other root exudates remained unidentified.

3.2. Effect of gluconate in the culture medium on enzymatic activity of gluconokinase and 6-phosphogluconate dehydrogenase of A. brasilense Cd

At six concentrations of gluconate added to culture medium for *A. brasilense* Cd, activity of the two enzymes showed different patterns. All concentrations of gluconate positively affected gluconokinase activity (Fig. 1b). In contrast, external gluconate had no effect on the activity of 6-phosphogluconate
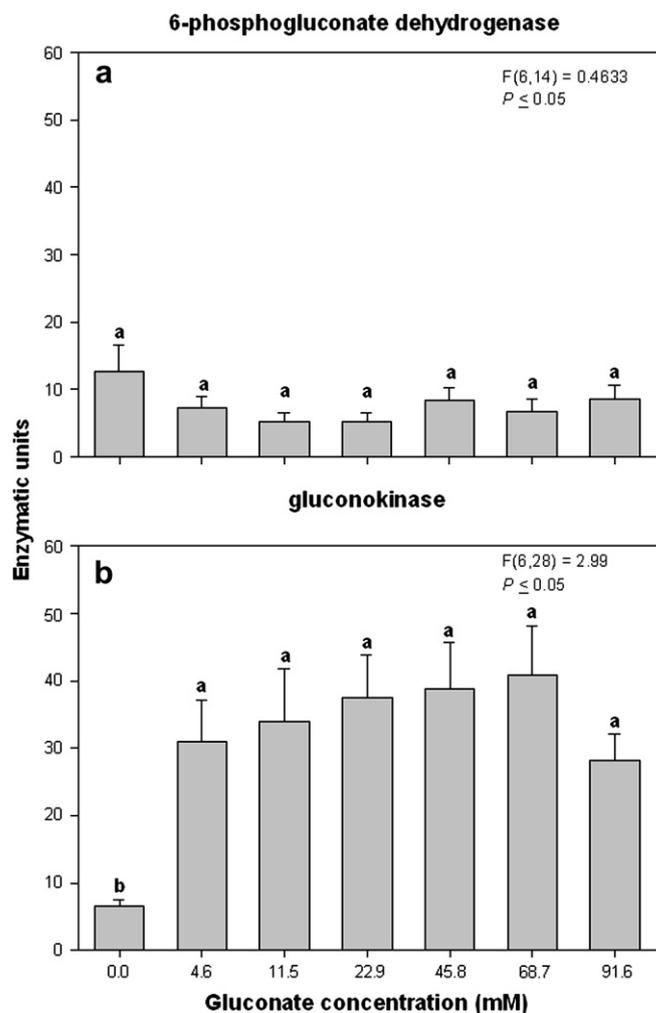

Fig. 1. Specific activity of the enzymes 6-phosphogluconate dehydrogenase (a) and gluconokinase (b) in cell extracts of *A. brasilense* Cd grown in TYG medium supplemented with six concentrations of gluconate. Enzymatic activity for both enzymes was defined as one enzyme unit equals Δ absorbance min^{-1} μg protein^{-1}. In each subfigure, columns denoted by a similar letter do not differ significantly at $P \leq 0.05$, using one-way ANOVA. Bars represent SE. Data are the mean of two identical experiments run separately.

dehydrogenase (Fig. 1a). This was also true for gluconokinase activity in the absence of gluconate in the medium.

3.3. Enzymatic activities in mesquite seedling roots, with and without inoculation with A. brasilense Cd

Evaluating the activity of both enzymes in mesquite roots at 15 and 23 days after inoculation with *A. brasilense* Cd revealed a different pattern for each enzyme, similar to the results of the culture medium studies. Inoculation did not enhance activity of both enzymes after 15 days of incubation (Fig. 2a,c). Gluconokinase activity, significantly increased in inoculated plants incubated for 23 days, but that of 6-phosphogluconate dehydrogenase did not change (Fig. 2b,d). Whether inoculated or not, activity of 6-phosphogluconate dehydrogenase in 23-day-old plants (8 days difference) was significantly higher (compare Fig. 2a and b), while increased gluconokinase activity
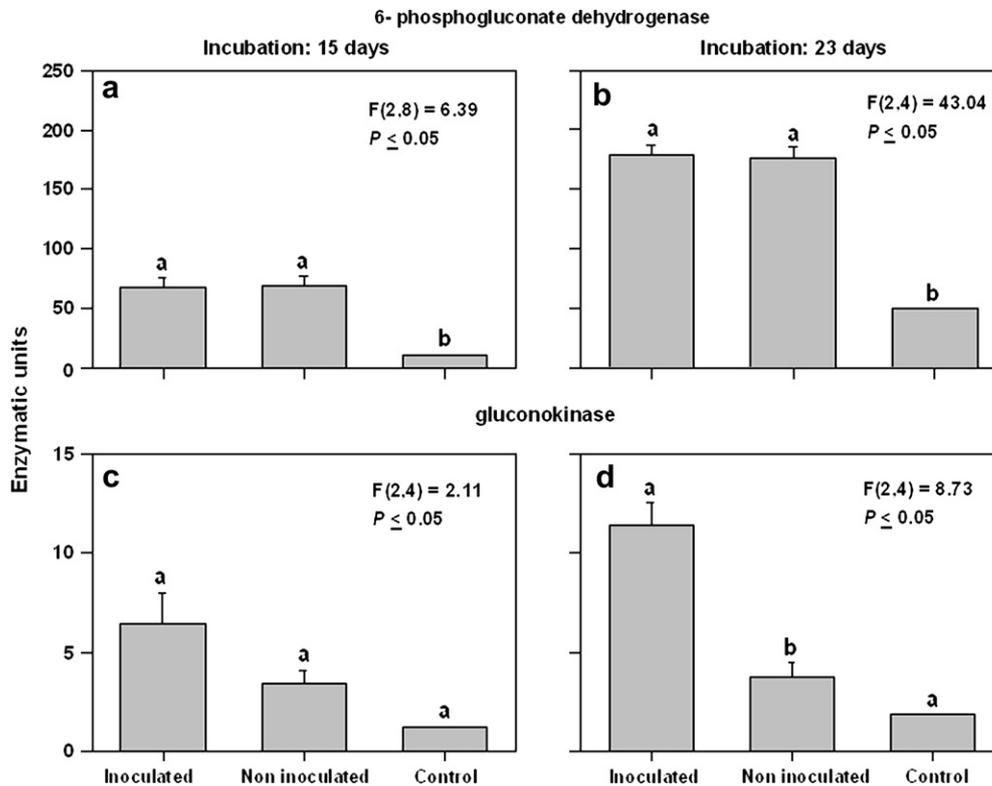
Fig. 2. Specific activity of the enzymes 6-phosphogluconate dehydrogenase (a,b) and gluconokinase (c,d) in mesquite seedling roots, with and without inoculation with *A. brasilense* Cd during two sampling periods. Enzymatic activity for both enzymes was defined as one enzyme unit equals Δ absorbance min^{-1} μg protein^{-1}. Controls were: a positive, 6-phosphogluconate dehydrogenase at 0.2 units of activity; a negative, a reaction mixture without an enzyme where the volume of the enzymatic extract was replaced by distilled water. In each subfigure, columns denoted by a similar letter do not differ significantly at $P \leq 0.05$, using one-way ANOVA. Bars represent SE. Data are the mean of two identical experiments run separately.

resulted only in inoculated treatments because older non-inoculated seedlings maintained the same activity as younger seedlings (compare Fig. 2c and d) and the sum of activities of the roots and the negative control was still lower than activity in inoculated plants.

3.4. Effect of inoculation with A. brasilense Cd on mesquite seedlings growing in hydroponics and sand cultures

In hydroponics, growth parameters of seedlings were height and dry weight (DW). DW (55.17 ± 10.27 mg plant^{-1}) varied greatly, as is common for slow-growing wild plants. Therefore, this parameter was not statistically significant. The height of inoculated plants increased significantly (14.6 ± 0.45 cm for inoculated plants, compared to 12.5 ± 0.9 cm for plants that were not inoculated (*t*-test, $P \leq 0.0005$, $n = 60$ plants, average of two trials) for the short duration of the experiment. Growth in sand substrate cultures was slower than in hydroponics. The most notable effects of inoculation were on plant dry weight (both of roots and shoots) and on total chlorophyll content, which significantly increased. Although the height of plants, number of leaves, and length of each leaf increased with time in the inoculated plants, and appeared greater than in non-inoculated plants, the short duration of the experiment did not allow detection of statistical differences between

inoculated and non-inoculated plants in these specific parameters. However, dry weight of roots and shoots and chlorophyll content significantly increased in inoculated plants (Table 1). Root colonization by *A. brasilense* Cd was very high and increased with time of incubation (Table 1).

4. Discussion

Desert plants, mainly cacti, benefit from plant growth-promoting bacteria of the genus *Azospirillum* [5,14,15,34]. The mechanism by which *Azospirillum* affects cacti and other desert plants is unknown, but is assumed to be similar to crop plants, mainly improving the plant hormone metabolism under unfavorable growth conditions and enhanced mineral uptake and water absorption [11]. The benefits from the plant root–bacteria association are presumed to be related to available root exudates. In this study, we investigated whether mesquite amargo trees can supply available root exudates to *A. brasilense* and whether two gluconate enzymes of the plant–bacteria interaction are active (or activated) during the association. This was done by evaluating exudation of gluconic acid, one of the most common growth substrates for *Azospirillum* [18,20] from mesquite roots and the activity level of two enzymes involved in its catabolism.

While gluconic acid is not a common root exudate [23], this study showed that mesquite amargo exudes this compound and

Table 1
Effect of inoculation of mesquite seedlings with *A. brasilense* Cd in sand and hydroponic cultures

Parameter	Inoculated		Non-inoculated	
	Root	Foliage	Root	Foliage
Dry weight of single plant after 40 days (mg)	$46 \pm 6.2a$	$71.4 \pm 12.4a$	$28.2 \pm 5.5b$	$64 \pm 5b$
Height after 26 days (mm)	NA	$42.2 \pm 1.9a$	NA	$41.6 \pm 1.3a$
Height after 40 days (mm)	NA	$61 \pm 3.3a$	NA	$63.45 \pm 3a$
Number of leaves after 26 days	NA	$2.58 \pm 0.19a$	NA	$2.5 \pm 0.19a$
Number of leaves after 40 days	NA	$5.37 \pm 0.44a$	NA	$4.89 \pm 0.4a$
Length of leaves after 26 days (mm)	NA	$32.1 \pm 3.1a$	NA	$26.9 \pm 2.6a$
Length of leaves after 40 days (mm)	NA	$69.9 \pm 6.9a$	NA	$65.4 \pm 7.3a$
Total chlorophyll content ($\mu g.g^{-1}$)	NA	$774.9 \pm 58.7a$	NA	$523.4 \pm 45.6b$
Root colonization after 15 days (cfu g dw^{-1})	$1.23 \pm 0.13 \times 10^9$	NA	0	NA
Root colonization after 24 days (cfu g dw^{-1})	$1.2 \pm 0.05 \times 10^{10}$	NA	0	NA

All data represent the average ($\pm$SE) of 20 plants grown in sand substrate cultures. Root colonization by *A. brasilense* Cd was evaluated in nine plants, in triplicate, grown in hydroponic units. NA, not applicable. Corresponding pairs of data in rows, denoted by different letters, differ significantly by Student's *t*-test at $P \leq 0.05$.

Azospirillum catabolizes it via two enzymes *in vitro* and perhaps *in planta*. We found that gluconokinase is induced by the presence of gluconic acid, while 6-phosphogluconate dehydrogenase is a constitutive enzyme. Although the concentration of root-exuded gluconate is smaller than the level of gluconate in bacterial cultures, the number of bacteria colonizing roots is very high and similar to medium cultures, demonstrating the ability of mesquite amargo trees to support populations of plant growth-promoting *A. brasilense*, even though this bacterium has, so far, not been detected in the natural rhizosphere of the tree. This level of colonization is similar to excellent root colonization reported for *Azospirillum* sp. for a few crops and is higher than root colonization by this species in most plant species where *Azospirillum* sp. exhibits growth promotion [11]. However, a reservation should be pointed out; although gluconate is the major root exudate in roots of mesquite amargo, other root exudates, although in small quantities and not identified in this study, may also contribute to the large proliferation of *A. brasilense* Cd in the roots.

There are no studies concerning gluconate enzymatic pathways in *Azospirillum*. However, in *Escherichia coli* and *Salmonella typhimurium*, the gene for gluconokinase is inducible [31,35–37]. Similar to our findings in *A. brasilense*, 6-phosphogluconate dehydrogenase of *E. coli* did not increase its activity when the concentration of gluconate in the medium increased [33]. Three explanations for this can be considered: (1) another enzyme in the gluconate pathways (6-phosphogluconate dehydratase) is using the same substrate as 6-phosphogluconate dehydrogenase (6-phosphogluconate) and both enzymes are active at the same time and this results in no increase in activity when the concentration of gluconate increases [33]; (2) there may be competition between 6-phosphogluconate dehydrogenase and 6-phosphogluconate dehydratase for the same substrate, 6-phosphogluconate [31]; (3) induction of 6-phosphogluconate dehydratase is favored over 6-phosphogluconate dehydrogenase under conditions of low oxygen concentration, as in *E. coli* during *in vitro* experiments [38]. Perhaps there is less oxygen around mesquite roots in hydroponic culture and this enzyme (not evaluated in this study) was more active than 6-phosphogluconate dehydrogenase, which was evaluated.

In summary, gluconic acid exudate from young mesquite seedling roots catabolized by *A. brasilense* Cd via two enzymes, gluconokinase and 6-phosphogluconate dehydrogenase. Roots inoculated with *A. brasilense* Cd exhibit significantly higher activity of gluconokinase, but not 6-phosphogluconate dehydrogenase.

References

[1] P. Felker, J.C. Guevara, Potential of commercial hardwood forestry plantations in arid lands-an economic analyses of *Prosopis* lumber production in Argentina and the United States, For. Ecol. Manage. 186 (2003) 271–286.

[2] J.L. León De La Luz, R. Domínguez Cadena, S.C. Díaz Castro, Evaluación del peso del leño a partir de variables dimensionales en dos especies de mezquite *Prosopis articulata* S. Watson y *P. palmeri* S. Watson, en Baja California Sur, México, Acta Bot. Mex. 72 (2005) 17–32.

[3] A. Carrillo-Garcia, J.-L. Leon de la Luz, Y. Bashan, G.J. Bethlenfalvay, Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran desert, Restor. Ecol. 7 (1999) 321–335.

[4] A. Carrillo-Garcia, Y. Bashan, G.J. Bethlenfalvay, Resource-island soils and the survival of the giant cactus, cardon, of Baja California Sur, Plant Soil 218 (2000) 207–214.

[5] A. Carrillo-Garcia, Y. Bashan, E. Diaz-Rivera, G.J. Bethlenfalvay, Effects of resource-island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran Desert, Restor. Ecol. 8 (2000) 65–73.

[6] H. Suzán-Azpiri, V.J. Sosa, Comparative performance of the giant cardon cactus (*Pachycereus pringlei*) seedlings under two leguminous nurse plant species, J. Arid Environ. 65 (2006) 351–362.

[7] M.B. Jenkins, R.A. Virginia, W.M. Jarrell, Ecology of fast-growing and slow-growing mesquite-nodulating rhizobia in Chihuahuan and Sonoron Desert ecosystems, Soil Sci. Soc. Am. J. 53 (1989) 543–549.

[8] N.H. Shoushtari, I.L. Pepper, Mesquite rhizobia isolated from the Sonoran Desert: physiology and effectiveness, Soil Biol. Biochem. 17 (1985) 797–802.

[9] Y. Bashan, E.A. Davis, A. Carrillo-Garcia, R.G. Linderman, Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran Desert, Agric. Ecosyst. Environ. Appl. Soil Ecol. 14 (2000) 165–176.

[10] D. Geesing, P. Felker, R.L. Bingham, Influence of mesquite (*Prosopis glandulosa*) on soil nitrogen and carbon development: implications for global carbon sequestration, J. Arid Environ. 46 (2000) 157–180.

[11] Y. Bashan, G. Holguin, L.E. de-Bashan, *Azospirillum*–plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003), Can. J. Microbiol. 50 (2004) 521–577.

[12] Y. Bashan, M. Moreno, E. Troyo, Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp, Biol. Fertil. Soils 32 (2000) 265–272.

[13] L.E. Gonzalez, Y. Bashan, Increased growth of the microalga *Chlorella vulgaris* when co-immobilized and co-cultured in alginate beads with the plant growth-promoting bacterium *Azospirillum brasilense*, Appl. Environ. Microbiol. 66 (2000) 1527–1531.

[14] M.E. Puente, Y. Bashan, Effect of inoculation with *Azospirillum brasilense* strains on the germination and seedlings growth of the giant columnar cardon cactus (*Pachycereus pringlei*), Symbiosis 15 (1993) 49–60.

[15] Y. Bashan, A. Rojas, M.E. Puente, Improved establishment and development of three cacti species inoculated with *Azospirillum brasilense* transplanted into disturbed urban desert soil, Can. J. Microbiol. 45 (1999) 441–451.

[16] P. Felker, D. Medina, C. Soulier, G. Velicce, M. Velarde, C. Gonzalez, A survey of environmental and biological factors (*Azospirillum* spp, *Agrobacterium rhizogenes*, *Pseudomonas aurantiaca*) for their influence in rooting cuttings of *Prosopis alba* clones, J. Arid. Environ. 61 (2005) 227–247.

[17] A. Vande Broek, P. Gysegom, O. Ona, N. Hendrickx, E. Prinsen, J. Van Impe, J. Vanderleyden, Transcriptional analysis of the *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene and identification of a *cis*-acting sequence involved in auxin responsive expression, Mol. Plant Microbe Interact. 18 (2005) 311–323.

[18] A. Hartmann, W. Zimmer, Physiology of *Azospirillum*, in: Y. Okon (Ed.), *Azospirillum*/Plant Associations, CRC Press, Boca Raton, FL, 1994, pp. 15–39.

[19] B. Rodelas, V. Salmeron, M.V. Martinez-Toledo, J. Gonzalez-Lopez, Production of amino acids by *Azospirillum brasilense* in chemically-defined medium amended with malate, gluconate or fructose for use as a nitrogen-fixation bacterial fertilizer inoculum, Soil Biol. Biochem. 26 (1994) 301–303.

[20] C.A. Westby, D.S. Cutshall, G.V. Vigil, Metabolism of various carbon sources by *Azospirillum brasilense*, J. Bacteriol. 156 (1983) 1369–1372.

[21] S. Shah, K.K. Rao, A. Desai, Production of catecholate type of siderophores by *Azospirillum lipoferum* M, Indian J. Exp. Biol. 31 (1993) 41–44.

[22] H. Rodriguez, T. Gonzalez, I. Goire, Y. Bashan, Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp, Naturwissenschaften 91 (2004) 552–555.

[23] G.G. Desbrosses, J. Kopka, M.K. Udvardi, *Lotus japonicus* metabolic profiling. Development of gas chromatography–mass spectrometry resources for the study of plant–microbe interactions, Plant Physiol. 137 (2005) 1302–1318.

[24] H.M. Alvarez, R.A. Silva, A.C. Cesari, A.L. Zamit, S.R. Peressutti, R. Reichelt, U. Keller, U. Malkus, C. Rasch, T. Maskow, F. Mayer, A. Steinbuchel, Physiological and morphological responses of the soil bacterium *Rhodococcus opacus* strain PD630 to water stress, FEMS Microbiol. Ecol. 50 (2004) 75–86.

[25] J.A. Simons, M.J. Teixeira De Mattos, O.M. Neijssel, Gluconate metabolism of *Klebsiella pneumoniae* NCTC 418 grown in chemostat culture, Arch. Microbiol. 159 (1993) 386–391.

[26] Y. Bashan, G. Holguin, R. Lifshitz, Isolation and characterization of plant growth-promoting rhizobacteria, in: B.R. Glick, J.E. Thompson (Eds.), Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, FL, 1993, pp. 331–345.

[27] D.R. Hoagland, D.I. Arnon, The water culture method for growing plants without soil, California Agricultural Experimental Station Circular 347 (1950) 347.

[28] Y. Bashan, J.-P. Hernandez, L.A. Leyva, M. Bacilio, Alginate microbeads as inoculant carrier for plant growth-promoting bacteria, Biol. Fertil. Soils 35 (2002) 359–368.

[29] Y. Bashan, L.E. de-Bashan, Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination, Soil Biol. Biochem. 37 (2005) 1795–1804.

[30] G. Mackinney, Absorption of light by chlorophyll solutions, J. Biol. Chem. 140 (1941) 315–322.

[31] D.G. Fraenkel, S.R. Levisohn, Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase, J. Bacteriol. 93 (1967) 1571–1578.

[32] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.

[33] I.N. Wang, D.E. Dykhuizen, Variation of enzyme activities at a branched pathway involved in the utilization of gluconate in *Escherichia coli*, Evolution 55 (2001) 897–908.

[34] M.E. Puente, C.Y. Li, Y. Bashan, Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings, Plant Biol. 6 (2004) 643–650.

[35] S.S. Cohen, Gluconokinase and the oxidative path of glucose 6-phosphate utilization, J. Biol. Chem. 189 (1951) 617–628.

[36] D.G. Fraenkel, B.L. Horecker, Pathways of D-glucose metabolism in *Salmonella typhimurium*. A study of a mutant lacking phosphoglucose isomerase, J. Biol. Chem. 239 (1964) 2765–2771.

[37] N. Peekhaus, T. Conway, What's for Dinner?: Entner–Doudoroff metabolism in *Escherichia coli*, J. Bacteriol. 180 (1998) 3495–3502.

[38] N.B. Coello, T. Istuúriz, The metabolism of gluconate in *Escherichia coli*: a study in continuous culture, J. Basic Microbiol. 32 (1992) 309–315.