

# *Stenocereus queretaroensis* a source of endophytic plant growth promoting bacteria for cropping *Zea mays*

## Abstract

Healthy growth of *Zea mays* requires  $\text{NH}_4\text{NO}_3$  as nitrogen fertilizer (NF), and its uptake is important to avoid loss of the NF. An alternative solution to enhance the root uptake capacity of *Z. mays* of NF at a dose to supply *Z. mays* demand without compromise its health; with beneficial endophytic genera and species of *Stenocereus queretaroensis* of the type *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus*. The objectives of this research were: a) to select from the interior of roots of *Stenocereus queretaroensis*: *B. vietnamiensis* and *G. diazotrophicus*, b) to analyze the growth of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* and NF at 50%. *B. vietnamiensis* and *G. diazotrophicus* were recovered from the roots of *S. queretaroensis* and inoculated on *Z. mays* seed with NF. Using the response variables: percentage of emergency, phenology and biomass to seedling and flowering, the experimental data were analyzed by ANOVA-Tukey ( $P \leq 0.05$ ). The percentage of emergency, phenology, and biomass at seedling and flowering of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* at 50% of  $\text{NH}_4\text{NO}_3$ , registered numerical values with statistical difference compared to those obtained in *Z. mays* without *B. vietnamiensis* and *G. diazotrophicus* only with NF at 100% or relative control (RC). This supports that *B. vietnamiensis* and *G. diazotrophicus*, endophytes of *S. queretaroensis*, invading the interior of *Z. mays* roots, converted metabolites related to root physiology into phytohormones that allowed maximum root uptake of  $\text{NH}_4\text{NO}_3$  at 50%.

**Keywords:** soil, xerophytic plant, domestic crop, chemical fertilization, endophytic bacteria

Volume 9 Issue 3 - 2022

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**Received:** April 24, 2022 | **Published:** May 24, 2022

## Introduction

The healthy growth of *Zea mays* (maize) demands  $\text{NH}_4\text{NO}_3$  as nitrogen fertilizer or NF.<sup>1,2</sup> This NF normally is not uptake by root system at 100% dose, due to several problem related with complex soil-root-microorganisms system in contrast not absorbed NF could caused loss fertility, or surface and groundwater contamination.<sup>3</sup> An ecological alternative solution to optimize reduced dose of the NF in *Z. mays* is to treat its seeds with: *Bacillus subtilis*,<sup>4</sup> *Paenibacillus polymixa*,<sup>5</sup> or *Pseudomonas putida*<sup>6</sup> including *Burkholderia cepacia*,<sup>7</sup> all of them are able to convert exudates organic compounds from the seed or/and the root into phytohormones,<sup>8,9</sup> to enhance the growth of an extensive and dense root system for reduced dose of NF.<sup>10,11</sup> In the literature it has been shown that some genera and species of those previously mentioned are specific for certain varieties of *Z. mays*, which can be relatively displaced by native soil rhizobacteria that colonize the roots of this cereal, without an evident beneficial effect on the uptake and optimization of NF reduced to 50% of what is recommended. An alternative solution to enhance and uptake NF at 50% is the selection of genera and species of endophytic bacteria that promote plant growth since they are competitive and effective in enhancing NF at 50%, without compromising healthy of *Z. mays*.<sup>12-14</sup> This endophytic bacteria can be to recover from xerophytes such as *Stenocereus queretaroensis* called “pitayo” in Spanish;<sup>15</sup> a cactus adapted to water stress due to low humidity, drastic changes in temperature; chemical related to the limitation of essential mineral salts of N (nitrogen),  $\text{PO}_4^{3-}$  (phosphates) and K (potassium): which includes biological ones such as root phytopathogens, given that *S. queretaroensis* grows successfully without human protection in this environment.<sup>16,17</sup> The hypothesis of this research is that certain endophytic genera and species such as *Burkholderia vietnamiensis*<sup>18,19</sup>

and *Gluconacetobacter diazotrophicus*<sup>20,21</sup> recovered from inside of the roots of *S. queretaroensis* could be beneficial for domestic crop such as *Z. mays*, in enhancing uptake of NF at 50% through *B. vietnamiensis* and *G. diazotrophicus*, by invading the root system of *Z. mays*, since they would avoid competition with other microorganisms in the rhizosphere and the soil, by living inside of root system to convert organic compounds into phytohormones,<sup>22-24</sup> which maximize the radical uptake capacity in NF reduced at 50%, and even that healthy growth of *Z. mays*. Therefore, the objectives of this research were: a) to select from inside *S. queretaroensis* roots: *B. vietnamiensis* and *G. diazotrophicus*, b) to analyze the growth of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* and the  $\text{NH}_4\text{NO}_3$  at 50%.

## Materials and methods

This research was carried out in the Environmental Microbiology Laboratory of the Biological Chemical Research Institute of the Michoacán University of San Nicolás de Hidalgo, the average microclimatic conditions were: temperature of 23.2°C, luminosity of 450  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , relative humidity of 67%.

## Origin and preparation of the soil

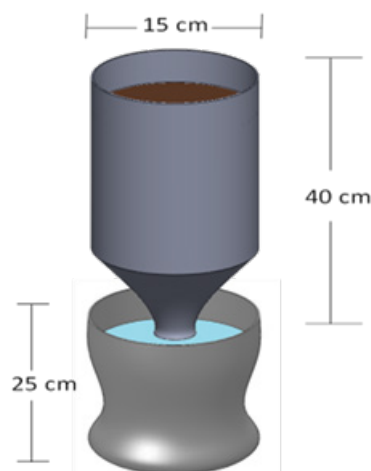
Table 1 shows the main soil properties of the experiment: one of the sodium lateritic type; Clay texture, poor in the concentration of organic matter with 4.57% and total N (nitrogen), with a slightly acidic pH of 6.64. This soil was collected from an agricultural plot called “La cajita” of the Tenencia Zapata in the municipality of Morelia, Mich., on km 5 of the Morelia-Pátzcuaro highway, México, solarized at 75°C/48 h to minimize pest problems and diseases.<sup>25</sup> Then a 1.0 Kg of soil was placed in the upper container of the Leonard jar, while in the lower part reservoir the NF or water, both parts were connected

by a 25 cm long cotton strip for capillarity movement of the liquid to the ground (Figure 1).

**Table 1** Physicochemical properties of the soil for the growth of *Zea mays* with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* endophytes of *Stenocereus queretaroensis*

Parameters	Values
Total, Nitrogen	0.62
Total, phosphorus	0.30
pH (1:20)	6.64
Organic matter (%)	4.57
Cation exchange capacity (C mol (+) Kg <sup>-1</sup> )	46.1
Texture (%)	20.56 (Cy)-37.8 (Si)-0.76(Sa)
Density (g/cm <sup>3</sup> )	2.01
Apparent density(g/cm <sup>3</sup> )	1.08
Porosity** (%)	46.35
Moisture saturation percentage (%)	46.95
Field capacity*** (%)	30.08
Useful humidity (%)	13.25

Sa, Sandy; Si, Silt; Cy, Clay; \*For soil of volcanic origin, \*\*Calculate from density and Apparent density; \*\*\* according texture type, + Reported for sandy loam soils NOM-021-RECNAT-2000.<sup>26</sup>



**Figure 1** Diagram of the Leonard jar. (Selection of endophytic *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* from roots of *Stenocereus queretaroensis*).

Isolates of endophytic *B. vietnamiensis* and *G. diazotrophicus* were recovered from inside the root of *S. queretaroensis*; 5-cm sections were taken and washed with sterile water, disinfected with 70% alcohol/2.5 min, then with 10% NaClO/2.5 min; then they were

macerated in a mortar with 10.0 mL of a saline solution (NaCl 0.85%) Roma™ detergent 0.01% (SSD), from which 1.0 mL was taken, and striked in *Pseudomonas cepacia* agar, azaleic acid with and without tryptamine (PCAAA) with the following composition (g□L-1): tryptamine 0.2; azaleic acid 2.0; K<sub>2</sub>HPO<sub>4</sub> 4.0; KH<sub>2</sub>PO<sub>4</sub> 4.0; yeast extract 0.02; MgSO<sub>4</sub> 0.2; pH adjusted to 6.7; PCAA was incubated at 28°C/72h; while to recover *G. diazotrophicus*, sucrose yeast extract agar (SYEA) was used with the following composition (g□L-1): sucrose 100.0; K<sub>2</sub>HPO<sub>4</sub> 2.0; KH<sub>2</sub>PO<sub>4</sub> 2.0; NaCl 1.0; MgSO<sub>4</sub> 3.0; yeast extract 1.0; 10 mL bromothymol blue at 2.0% (p/v)<sup>27</sup> (Cavalcante & Dobereiner, 1988) with 10 mL/L of the trace element solution with the following composition (g□L-1): H<sub>3</sub>BO<sub>3</sub> 2.86; ZnSO<sub>4</sub> 0.22; MnCl<sub>2</sub> 1.81; K<sub>2</sub>MnO<sub>4</sub> 0.09; the pH was adjusted to 5.7; both PCAA and SYEA were incubated at 30°C/72h;<sup>27</sup> when round bright translucent colonies suspicious for *B. vietnamiensis* were detected, they were again growth in PCAA to obtain an axenic culture, then short Gram-negative bacilli were registered by Gram stain; microscopic morphological characteristics common in *B. vietnamiensis*: while for *G. diazotrophicus* in the SYEA, bulging and bright round colonies with a yellow intracellular pigment were observed, while Gram-negative coccobacilli were detected under the microscope, which according to the literature belong to these genus.<sup>21,28-30</sup>

Growth of *Zea mays* with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* endophytes and 50% of NF. *Z. mays* seeds were disinfected with NaClO at 3% (v/v)/5 min, washed six times with sterile water, then with alcohol at 70% (v/v)/5 min, washed six times with sterile water, later in plastic bags of 250 g for every 10 *Z. mays* seeds, were inoculated with 1.0 mL of *B. vietnamiensis* and *G. diazotrophicus* in a concentration that was adjusted to 1 x 10<sup>6</sup> CFU/mL, obtained by viable count on plate in PCAA and SYEA; when both were inoculated, a 1:1 ratio was used; after the inoculation of the seeds with *B. vietnamiensis* and *G. diazotrophicus* were shaken for 1 h to ensure the entry of *B. vietnamiensis* and *G. diazotrophicus* to the seeds of *Z. mays*, then five seeds of cereals were sown in each Leonard Jar. *Z. mays* with or without *B. vietnamiensis* and *G. diazotrophicus*; according to the experimental design in Table 2, randomized blocks with two controls, three treatments and six replicates: *Z. mays* without *B. vietnamiensis* and *G. diazotrophicus* irrigated only with water or absolute control (AC); *Z. mays* without *B. vietnamiensis* and *G. diazotrophicus* fed with the NH<sub>4</sub>NO<sub>3</sub> at 100% or relative control (RC); *Z. mays* inoculated individual/in consortium with *B. vietnamiensis* and *G. diazotrophicus* with NF at 50% in a mineral solution for this cereal with the following chemical composition (g□L-1): NH<sub>4</sub>NO<sub>3</sub> 12.0, KH<sub>2</sub>PO<sub>4</sub> 3.0, K<sub>2</sub>HPO<sub>4</sub> 3.5, MgSO<sub>4</sub> 1.5, CaCl<sub>2</sub> 0.1, FeSO<sub>4</sub> 0.5 mL/L, and a 1.0 mL/L trace element solution, pH adjusted to 7.0, in distilled water, while NH<sub>4</sub>NO<sub>3</sub> or NF was reduced to 50% equivalent to 6 g□L-1.<sup>25</sup>

**Table 2** Experimental design to analyze the growth of *Zea mays* with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* endophytes from root of *Stenocereus queretaroensis* with NH<sub>4</sub>NO<sub>3</sub> at 50%

*Treatments + <i>Zea mays</i>	<i>Burkholderia vietnamiensis</i>	<i>Gluconacetobacter diazotrophicus</i>	water	NH <sub>4</sub> NO <sub>3</sub> as nitrogen fertilizer
Absolute control (AC) irrigated only water	-	-	+	-
Relative control fed(RC) NH <sub>4</sub> NO <sub>3</sub> at 100%	-	-	-	100%
Treatment 1	+	-	-	50%
Treatment 2	-	+	-	50%
Treatment 3	+	+	-	50%

+n=6; (+) = applied; (-) = no applied.

The growth response variables of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* were: the percentage of emergence (%), at the seedling and flowering level based on phenology: plant height (PH) and root length (RL); in the biomass: aerial fresh weight (AFW) and radical (RFW); with aerial dry weight (ADW) and radical (RDW). The experimental data obtained were subjected to ANOVA analysis of variance using Tukey's comparative test of means ( $P \leq 0.05$ ), for which the statistical program Statgraphics Centurion<sup>31</sup> was used.

Evidence of the presence of *B. vietnamiensis* and *G. diazotrophicus* in the stem and roots of *Zea mays*

The sensitivity profile of *B. vietnamiensis* and *G. diazotrophicus* was obtained before and after inoculating *Z. mays*. Using the Kirby and Bauer method for genera and species of Gram negative bacteria: Ampicillin, Cefotaxime, Ceftazidime, Cefuroxime, Pefloxacin, Tetracycline and Trimethoprim-Sulfamethoxazole, Cephalotin, Dicloxacilin, Erythromycin, Gentamicin and Penicillin.<sup>32</sup> Both *B. vietnamiensis* and *G. diazotrophicus* isolated from *S. queretaroensis* were grown on nutrient agar and on PCAA and SYEA; As a reference, the sensidiscs were placed before inoculating the *Z. mays* seed, then they were incubated at 32°C/48 h, and the inhibition halos were measured according to the Kirby-Bauer method. Later, when *Z. mays* was inoculated with *B. vietnamiensis* and *G. diazotrophicus* and the cereal reached the level of seedling and flowering, the following were taken: 1.0 g of leaves, stem and/or roots each were disinfected and macerated as described in item b), then 1.0 mL of leaves, stem and root with *B. vietnamiensis* and *G. diazotrophicus*; it was grown with a sterile swab in nutrient agar, PCAA and SYEA, then the antibiotic sensidiscs were placed and incubated from 24 to 48 h/30°C. Inhibition halos were then measured according to the Kirby-Bauer method,<sup>33,34</sup> and the sensitivity pattern of individual *B. vietnamiensis* and *G. diazotrophicus* was obtained to compare with the antibiotic sensitivity profile of each before inoculating the seed of *Z. mays*. Finally, to confirm that they belonged to *B. vietnamiensis* and *G. diazotrophicus*, specific biochemical tests were used to identify them as members of these bacterial groups.<sup>27,31,35,36</sup>

## Results and discussion

Table 3 shows the results of the emergence percentage of the *Z. mays* seeds treated with *B. vietnamiensis* and *G. diazotrophicus* and  $\text{NH}_4\text{NO}_3$  at 50%, with 95% of emergence, which indicates that *B. vietnamiensis* and *G. diazotrophicus* converted exudates from the seed, known as spermosphere effect, such as tryptophan into phytohormons to accelerate starch hydrolysis, to end embryo dormancy and to accelerate the rate of stem and root primordium emergence, as detected in *Z. mays* only treated with *B. vietnamiensis* or with *G. diazotrophicus* and FN at 50%.<sup>9,37</sup> These percent emergence values were statistically different than 84% *Z. mays* without *B.*

*vietnamiensis* or either *G. diazotrophicus* fed only of the NF at 100% used as relative control (RC).

**Table 3** Percentage of emergence of *Zea mays* seeds treated with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* and  $\text{NH}_4\text{NO}_3$  at 50%

*Tratamiento (T) +Zea mays	Emergency percentage (%)
(AC) absolute control irrigated only water	81 <sup>b**</sup>
(RC) relative control fed $\text{NH}_4\text{NO}_3$ at 100%	84 <sup>b</sup>
(T1) <i>B. vietnamiensis</i> $\text{NH}_4\text{NO}_3$ at 50%	92 <sup>a</sup>
(T2) <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	89 <sup>b</sup>
(T3) <i>B. vietnamiensis</i> and <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	95 <sup>a</sup>

\*n= 6, \*\*Values with different letters indicate statistical difference according to ANOVA-Tukey  $P \leq 0.05$ ).

In Table 4, the growth of *Z. mays* with *G. diazotrophicus* and 50% NF is presented for seedling, where 17.5 cm of PH and 13.0 cm of RL were registered. Numerical values without statistical difference with those observed in *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* plus NF at 50%, according to various reports it is shown that these endophytic genera interact efficiently with wild hosts, compared with those that inhabit the phyllosphere and/or the rhizosphere of a wide variety of domestic plants.<sup>38</sup> What supports, *B. vietnamiensis* and *G. diazotrophicus* after germination colonized and penetrated the roots of *Z. mays* through different mechanisms some known and other not yet discovered that occur during plant,<sup>39</sup> inside the plant conduction system they converted some low molecular weight carbon compounds derived from the metabolism of photosynthesis into phytohormones,<sup>40</sup> which accelerated and improved the functioning of the root system to maximize uptake of NF to 50%, without compromising the healthy growth of *Z. mays*.<sup>19,20</sup> shown in an increase in the numerical values of the phenology that were statistically different from those registered when *Z. mays* grew without *B. vietnamiensis* and *G. diazotrophicus* and the NF at 100% or relative control (RC); which the following results 13.3 cm PH and 9.1 RL. The growth of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* and the NF at 50% based on the biomass registered 1.4 g of AFW and 0.37 g of RFW, while 0.23 g of DAW and 0.09 g DRW, the above indirectly demonstrates that both *B. vietnamiensis* and *G. diazotrophicus* were inside the roots, in the conduction system, transformed compounds from photosynthesis into phytohormones, to induce the greatest uptake of  $\text{NH}_4\text{NO}_3$  and to optimize the dose at 50%.<sup>12,24,41</sup> These numerical values of the biomass were statistically different with the 0.9 g of AFW and the 0.24 g of RFW, with the 0.08 g of DAW and with the 0.02 g of DRW of *Z. mays* used as RC, which shows that based on the healthy growth of *Z. mays*, the positive effect of *B. vietnamiensis* and *G. diazotrophicus* in optimizing  $\text{NH}_4\text{NO}_3$  at 50%.

**Table 4** Growth of *Zea mays* with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* in phenology and seedling biomass with  $\text{NH}_4\text{NO}_3$  at 50%

*Treatment(T) +Zea mays	Plant height (cm)	Radical length (cm)	Fresh weight (g)		Dry weight (g)	
			Aerial	Radical	Aerial	Radical
(AC) absolute control irrigated only water	12.5 <sup>**</sup>	8.9 <sup>c</sup>	0.7 <sup>c</sup>	0.19 <sup>c</sup>	0.04 <sup>c</sup>	0.02 <sup>c</sup>
(RC) relative control fed $\text{NH}_4\text{NO}_3$ at 100%	13.3 <sup>b</sup>	9.1 <sup>b</sup>	0.9 <sup>b</sup>	0.24 <sup>b</sup>	0.08 <sup>c</sup>	0.02 <sup>c</sup>
(T1) <i>B. vietnamiensis</i> and $\text{NH}_4\text{NO}_3$ at 50%	14.9 <sup>a</sup>	9.6 <sup>b</sup>	1.0 <sup>b</sup>	0.29 <sup>b</sup>	0.14 <sup>b</sup>	0.07 <sup>a</sup>
(T2) <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	17.5 <sup>a</sup>	13.0 <sup>a</sup>	1.6 <sup>a</sup>	0.40 <sup>a</sup>	0.17 <sup>b</sup>	0.05 <sup>b</sup>
(T3) <i>B. vietnamiensis</i> and <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	15.7 <sup>a</sup>	12.8 <sup>a</sup>	1.4 <sup>a</sup>	0.37 <sup>a</sup>	0.23 <sup>a</sup>	0.09 <sup>a</sup>

\*n=6; \*\*Values with different letters indicate a statistical difference according to ANOVA-Tukey ( $P \leq 0.05$ ).



Table 5 shows the growth of *Z. mays* with *B. vietnamiensis* individually or in combination with *G. diazotrophicus* and 50% of  $\text{NH}_4\text{NO}_3$  flowering; there, they registered 92.17 cm of PH and 28.12 cm of RL; while in *Z. mays* with *G. diazotrophicus* and 50%, of  $\text{NH}_4\text{NO}_3$ , 90.93 cm AP and 26.95 cm RL were obtained. These numerical data indicate that *B. vietnamiensis* and *G. diazotrophicus*, after colonizing inside of the root system, transformed organic compounds from root physiology into phytohormones,<sup>22</sup> inducing a dense and extensive root system, the roots of *Z. mays* had a higher exploration capacity to uptake  $\text{NH}_4\text{NO}_3$  at 50% without compromising the healthy growth of *Z. mays*.<sup>19,23,42</sup> The numerical values of the phenology of *Z. mays* treated with *B. vietnamiensis* and *G. diazotrophicus* were statistically different compared to the 74.41 cm RL and 21.05 cm RL of *Z. mays* without *B. vietnamiensis* and *G. diazotrophicus* and the NF at 100% or relative control (RC). Concerning the biomass of the growth of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* and the NF at

50%, they registered 31.03 g of AFW and 8.91 g of DRW, as well as 5.97 g of DAW and 1.23 g DRW; like *Z. mays* with *G. diazotrophicus* and NF at 50%. This suggests that both *B. vietnamiensis* and *G. diazotrophicus* endophytes of *S. queretaroensis*, by invading the interior of the roots of *Z. mays*, converted metabolites related to root physiology into phytohormones that induced the maximum radical uptake for the optimization of NF at 50%.<sup>41,43,44</sup> These numerical values of the biomass of *Z. mays* with *B. vietnamiensis* and *G. diazotrophicus* and the NF at 50% were statistically different with the 26.06 g of AFW and the 2.85 g of RFW, with the 2.91 g of ADW and with the 0.91 g of DRW from *Z. mays* used as RC, that fact suggests that *B. vietnamiensis* as *G. diazotrophicus* when they are inside the root transformed organic compounds producing during root physiology into phytohormones that activated and improved the capacity of the root system for maximum uptake and optimization of  $\text{NH}_4\text{NO}_3$  at 50%, and even that the healthy growth of *Z. mays*.<sup>15,19,21</sup>

**Table 5** Growth of *Zea mays* at flowering stage with *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* in phenology and biomass and  $\text{NH}_4\text{NO}_3$  at 50%

*Treatments (T) * <i>Zea mays</i>	Plant height (cm)	Radical length (cm)	Fresh weight (g)		Dry weight (g)	
			Aerial	Radical	Aerial	Radical
(AC) absolute control irrigated only water	71.13 <sup>ab</sup>	20.03 <sup>b</sup>	21.02 <sup>b</sup>	2.55 <sup>b</sup>	2.08 <sup>c</sup>	0.45 <sup>c</sup>
(RC) relative control fed $\text{NH}_4\text{NO}_3$ at 100%	74.41 <sup>b</sup>	21.05 <sup>b</sup>	22.97 <sup>b</sup>	2.85 <sup>b</sup>	2.91 <sup>b</sup>	0.41 <sup>c</sup>
(T1) <i>B. vietnamiensis</i> $\text{NH}_4\text{NO}_3$ at 50%	88.43 <sup>a</sup>	24.13 <sup>a</sup>	26.09 <sup>b</sup>	6.7 <sup>a</sup>	3.93 <sup>b</sup>	0.91 <sup>b</sup>
(T2) <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	90.93 <sup>a</sup>	26.95 <sup>a</sup>	29.73 <sup>a</sup>	7.93 <sup>a</sup>	4.19 <sup>a</sup>	1.07 <sup>a</sup>
(T3) <i>B. vietnamiensis</i> and <i>G. diazotrophicus</i> $\text{NH}_4\text{NO}_3$ at 50%	92.17 <sup>a</sup>	28.12 <sup>a</sup>	31.03 <sup>a</sup>	8.91 <sup>a</sup>	5.97 <sup>a</sup>	1.23 <sup>a</sup>

\*n=6; \*\*Values with different letters had a statistical difference according to ANOVA-Tukey (P<0.05).

In Table 6, the sensitivity profile of *B. vietnamiensis* and *G. diazotrophicus* endophytes of *S. queretaroensis* is shown to demonstrate that healthy root, stem and leaf growth of *Z. mays* seedling and flowering level, according to the Kirby and Bauer method, registered sensitivity to: Cefotaxime, Tetracycline, Trimethoprim-Sulfamethoxazole, Cefalotin, Erythromycin, in contrast resistant to: Ampicillin, Cefazidime, Cefuroxime, Pefloxacin, Gentamicin, and

Penicillin. This sensitivity profile was compared to that obtained for *B. vietnamiensis* and *G. diazotrophicus* before inoculating *Z. mays* seed. This suggests that *B. vietnamiensis* and *G. diazotrophicus* invaded the xylem of *Z. mays* to transform organic compounds from root physiology into phytohormones,<sup>44,45</sup> which allowed the maximum uptake of NF to 50% for healthy growth.<sup>46</sup>

**Table 6** Antibiotic sensitivity profile of *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* recovered from roots, stem and leaf of *Zea mays*  $\text{NH}_4\text{NO}_3$  at 50%

Antibiotic	Endophytic isolates from <i>Stenocereus queretaroensis</i>		Recover from <i>Zea mays</i>					
	<i>B. vietnamiensis</i>	<i>G. diazotrophicus</i>	<i>B. vietnamiensis</i>			<i>G. diazotrophicus</i>		
			Root	stem	leaf	root	stem	leaf
Ampicillin	-	-	-	-	-	-	-	-
Cefotaxima	+	+	+	+	+	+	+	+
Ceftazidime	-	-	-	-	-	-	-	-
Cefuroxime	-	-	-	-	-	-	-	-
Pefloxacin	-	-	-	-	-	-	-	-
Tetracycline	+	+	+	+	+	+	+	+
Trimetoprim-Sulfamethoxazole	+	+	+	+	+	+	+	+
Cefalotin	+	+	+	+	+	+	+	+
Dicloxacin	-	-	-	-	-	-	-	-
Erythromicin	+	+	+	+	+	+	+	+
Gentamicin	-	-	-	-	-	-	-	-
Penicillin	+	+	+	+	+	+	+	+

(+) = sensitivity; (-) = resistant.

Table 7 shows the biochemical profile of *B. vietnamiensis* and *G. diazotrophicus* endophytes of *S. queretaroensis*, based on a prototype strain and related literature; there, it is reported that the genus and species of *B. vietnamiensis* is a short bacillus Gram negative, a

facultative aerobe that synthesizes catalase and oxidase; so it has an oxidative metabolism, it uses sugars: esculin, glucose, which do not ferment, but organic acids: sodium citrate; it is a solubilizer of  $\text{PO}_4^{-3}$  by the generation of acid and alkaline phosphatases; They reduce  $\text{NO}_3$

to NO<sub>2</sub>. *B. vietnamiensis* fixes N<sub>2</sub>, grows in a mineral medium without any form of combined N;<sup>47,48</sup> selectively uses tryptamine as a source of N; convert tryptophan into indole acetic acid and induces a dense and larger root system, which allowed *Z. mays* to uptake NH<sub>4</sub>NO<sub>3</sub> at 50%.<sup>5,49,50</sup> *Gluconacetobacter diazotrophicus* of *S. queretaroensis* is an osmophilic endophytic genus and species similar to the prototype strain of *G. diazotrophicus* of *S. officinarum* (sugar cane). It has an oxidative metabolism of sugars: glucose, fructose and sucrose; uses citrate as the sole source of carbon and energy; by glucose fermentation it generates ethanol, lactate and acetic acid;<sup>50</sup> fixes N<sub>2</sub> in facultative endophyte association, grows in a culture medium without any source of organic or inorganic N, in anaerobiosis reduces NO<sub>3</sub> to NO<sub>2</sub>; transform L-tryptophan into auxin, a phytohormone that generates a dense radical system that in *Z. mays* improved radical uptake of NH<sub>4</sub>NO<sub>3</sub> and optimized the dose reduced at 50%.<sup>52-54</sup>

**Table 7** Biochemical profile of *Burkholderia vietnamiensis* and *Gluconacetobacter diazotrophicus* endophytes of *Stenocereus queretaroensis* in *Zea mays* and nitrogen fertilizer at 50%

Biochemical test	<i>Burkholderia vietnamiensis</i>		<i>Gluconacetobacter diazotrophicus</i>	
	*Prototype	**Endo phytic isolated	*Proto type	**Endo phytic isolated
Gram stain	-	-	-	-
Rod shape	short	short	cocco bacilli	coco bcilli
Esculin hydrolisis	+	+	+	+
Glucose fermentation	-	-	+	+
Vogues-Proskauer	-	-	+	+
Xilose	+	+	+	+
Sodium citrate	+	+	+	+
Indole	+	+	+	+
Urease	+	+	-	-
arginine hydrolase	+	+	+	+
Nitrate reduction	+	+	+	+

\*Prototype strain response. \*\*Recovered from inside of *S. queretaroensis*; positive reaction (+); negative reaction (-).

## Conclusions

This research shows that *S. queretaroensis*, a wild plant from desert environments, could be a source of plant growth promoting endophytic bacteria like *B. vietnamiensis* and *G. diazotrophicus*. These bacteria interact with domestic crops, such as *Z. mays*, to improve nitrogen uptake for healthy growth, prevent soil and productivity loss, and environmental pollution from hyperfertilization during *Z. mays* cropping.

## Acknowledgments

We acknowledge support to the Project 2.7 (2022) from CIC-UMSNH also to Phytonutrientes de México and BIONUTRA, S.A de CV Maravatio, Michoacán, México.

## Conflicts of interest

The authors state that there is no conflict of interest.

## Funding

To Project 2.7 (2022) from CIC-UMSNH and to Phytonutrientes de México and BIONUTRA, S.A: de CV Maravatio, Michoacán, México.

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