**Native isolates of *Gluconacetobacter diazotrophicus* solubilizes phosphates and promotes plant growth in *Solanum lycopersicum* L.**

**Aislamientos nativos de *Gluconacetobacter diazotrophicus* solubilizan fosfatos y promueven el crecimiento vegetal en *Solanum lycopersicum* L.**

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**ABSTRACT**

*Solanum lycopersicum* L. is one of the most important crops worldwide with potential for sustainable production through the use of plant growth promoting rhizobacteria such as *Gluconacetobacter diazotrophicus,* due to its phosphate solubilization capacity.This study aimed to determine the ability of two native isolates of *Gluconacetobacter diazotrophicus* from Colombian agroecosystems to solubilize inorganic phosphate and promote the growth of *Solanum lycopersicum* L. in seedling stage.The work was carried out in two stages. In a first stage, the solubilization of phosphorus (P) was measured from three sources of inorganic phosphate (Ca3(PO4)2, AlPO4 and FePO4) in solid NBRIP medium and in submerged cultures. In the second stage, inoculation of tomato seeds was performed with isolates and type strain in the presence and absence of phosphorus fertilizer. The native isolates had the ability to solubilize the three sources of inorganic P, both in solid media and in submerged cultures. Isolates of *G. diazotrophicus* GIBI025, GIBI029 and type strain ATCC 49037 achieved a reduction in the days to cotyledon of 18.92%, 13.36% and 9.74% respectively. Isolate GIBI025 stood out with yields on variables related to the promotion of plant growth evaluated equal to or above the control with phosphorus fertilizer, demonstrating the potential of the isolates in promoting the growth of *Solanum lycopersicum* L.

**KEY WORDS**: Biofertilization; Bioprospecting; *Gluconacetobacter diazotrophicus*; Organic and inorganic source; Plant-bacteria interaction; Plant growth promoting rhizobacteria; Phosphorus solubilization; Phosphate solubilizing microorganisms; Seedlings tomato; Submerged cultures.

**RESUMEN**

*Solanum lycopersicum* L. es uno de los cultivos más importantes a nivel mundial con potencial de producción sostenible mediante el uso de rizobacterias promotoras del crecimiento vegetal como *Gluconacetobacter diazotrophicus*, debido a su capacidad de solubilización de fosfatos. Este estudio tuvo como objetivo determinar la capacidad de dos aislamientos nativos de *Gluconacetobacter diazotrophicus* de agroecosistemas colombianos para solubilizar fosfato inorgánico y promover el crecimiento de plántulas de *Solanum lycopersicum* L. El trabajo se llevó a cabo en dos etapas. En una primera etapa, se midió la solubilización del fósforo (P) a partir de tres fuentes de fosfato inorgánico (Ca3 (PO4)2, AlPO4 y FePO4) en medio NBRIP sólido y en cultivos sumergidos. En la segunda etapa, se realizó la inoculación de semillas de tomate con aislamientos y cepa patrón en presencia y ausencia de fertilizante de fósforo. Los aislados nativos tuvieron la capacidad de solubilizar las tres fuentes de P inorgánico, tanto en medios sólidos como en cultivos sumergidos. Los aislamientos de *G. diazotrophicus* GIBI025, GIBI029 y la cepa tipo ATCC 49037 lograron una reducción de los días a cotiledón de 18,92%, 13,36% y 9,74% respectivamente. El aislamiento GIBI025 se destacó con rendimientos en variables relacionadas con la promoción del crecimiento vegetal evaluados iguales o superiores al control con fertilizante de fósforo, demostrando el potencial de los aislados en promover el crecimiento de *Solanum lycopersicum* L.

**PALABRAS CLAVES**: Biofertilización; Bioprospección; Cultivos sumergidos; *Gluconacetobacter diazotrophicus*; Fuente orgánica e inorgánica; Interacción planta-bacteria; Microorganismos solubilizadores de fosfato; Plántulas de tomate; Rizobacterias Promotoras del Crecimiento Vegetal; Solubilización de fósforo.

**INTRODUCTION**

*Solanum lycopersicum* L. is one of the most important crops worldwide (FAOSTAT, 2019); however, to achieve high yields in production, it is required the use of large amounts of agrochemicals for crop nutrition and pest control. These practices increase production costs and promote environmental pollution (Shang *et al*., 2019).

Phosphorus (P) is one of the most important macronutrients for plant development. It is present as a constituent of macromolecular structures and as a metabolite involved in energy transfer, protein activation, cell division, photosynthesis, regulation of metabolic processes, among others (de Bang *et al*., 2021).

In soil, P concentration usually ranges from 100-3000 mg kg-1 (Sánchez, 2019); however, only 0,1% of total P exists in soluble form available to plants (Alewell *et al*., 2020), making it a limiting factor in their development.

An important part of the P present in agricultural soils derives from chemical fertilization. Approximately 70% of the P coming from the use of agrochemicals is found in the soil as insoluble complexes: calcium phosphate, aluminum phosphate, phosphate ion (Mitter *et al.*, 2021).

P solubilizing microorganisms can solubilize and mineralize P from inorganic and organic sources in the soil (Rafi *et al*., 2019) and can be used as inoculants to increase the bioavailability of this element for plants, enabling the implementation of sustainable agriculture (Billah *et al*., 2019). The mechanisms involved in microbial solubilization of the different forms of insoluble phosphate include processes of acidification, chelation, exchange reactions, production of organic acids and enzymatic action (Kafle *et al*., 2019).

*Gluconacetobacter diazotrophicus* is an endophytic bacterium with the ability to promote growth in different crops of economic importance (Tufail *et al.*, 2021). Cavalcante and Döbereiner (1988) first isolated it and showed that it could fix atmospheric nitrogen in a non-associative form and, since then, there are numerous reports supporting its role in the supply of nitrogen to plants. Other studies have reported that *G. diazotrophicus* can promote plant growth by other mechanisms of action, among which are the production of phytohormones (Kudoyarova *et al*., 2019), antagonism against pathogens (Rodriguez *et al.*, 2019) and solubilization of minerals such as zinc and P (Paredes-Villanueva *et al*., 2020).

With regard to microbial solubilization of phosphates, there are numerous reports showing the activity of solubilizing microorganisms *in vitro* and, in almost all of such works, solubilization of tricalcium phosphate (TCP) in solid NBRIP medium or Pikovskaya (Li *et al.*, 2019) is used as a selection criterion. However, it has been observed that the strains selected under this single criterion, when tested in assays in plants, do not exhibit good activity. It is therefore necessary to use different methodologies for the selection of microorganisms, including the use of different sources of phosphate and conducting *in vivo* assays.

This work aims to characterize indigenous isolates of *Gluconacetobacter diazotrophicus* regarding solubilization capacity of different sources of P in *S. lycopersicum* seedlings.

**METHOD**

This study was carried out in the laboratories of the Institute for Research in Microbiology and Agroindustrial Biotechnology of the Catholic University of Manizales and in the Tesorito Farm of the University of Caldas (Manizales, Colombia), located in the rural area of Manizales (Colombia) at an altitude of 2.340 masl (5° 01' 49" N y -75° 26' 13" W), with annual rainfall of 1.800 mm, relative humidity of 78%, solar brightness of 1,215 h-light per year, average temperature of 17,5 °C and type of sandy loam soil (Universidad de Caldas, 2014).

**Microbial cultures**

Two native isolates of *G. diazotrophicus* identified as GIBI025 and GIBI029 from the Collection of Microorganisms of the Catholic University of Manizales were used, isolated and identified in previous works (Restrepo *et al*., 2017). Type strain ATCC 49037 from the NCIMB (National Collection of Industrial, Food and Marine Bacteria) was used. The bacteria were recovered from vials cryopreserved at -80 °C in Potato Dextrose Agar (PDA) Oxoid® at a temperature of 30 °C to obtain isolated colonies.

## Evaluation of phosphate solubilizing capacity

## An inoculum of *G. diazotrophicus* from a colony isolated in PDA was obtained and transferred to vials containing 5 mL of DYGS broth (Alexander *et al*., 2020). Later, a scaling was performed to a volume of 250 mL in the same culture medium. The broths were incubated for four to five days, at 150 rpm and 30 °C (Actum, Colombia) to an approximate concentration of 108 cells/mL (D.O 600nm 0,9-1,0) determined in a spectrophotometer (Thermo Bio 5, USA).

**Semiquantitative evaluation**

The assembly was performed out in Petri dishes of 90 mm in diameter containing NBRIP medium (Nautiyal, 1999) supplemented individually with 5 g/L of different forms of inorganic P (Ca3(PO4)2, AlPO4 and FePO4). Bromocresol green dye at a concentration of 0,022 g/l was added as pH indicator to facilitate the display of solubilization halos. Of each bacterium were planted 20 µ/L of the inoculum on the surface of each medium in triplicate. The Petri dishes were incubated at 30 °C for 10 days, period during which daily monitoring was made and the Solubilization Index (SI) was calculated according to the Equation 1, where A corresponds to the total diameter (colony + halo) and B to the diameter of the colony according to Edi–Premono *et al*., (1996). An estimate of the amount of acids released by the bacteria was performed by measuring the size of the color changing halos given by the presence of the pH indicator, and the Acid Production Index (API) was calculated based on the modified Edi-Premono *et al*., formula (1996). A: total diameter of the colony + color changing halo, and B: size of the colony.

$$SI=\frac{A}{B}$$

(Eq. 1)

**Quantitative evaluation**

Submerged cultures of *G. diazotrophicus* were made adding 25 mL of the bacterial inoculum to bioreactors (500 mL Erlenmeyer) containing 225mL of NBRIP medium (Nautiyal, 1999) supplemented independently with Ca3(PO4)2, AlPO4 and FePO4. The cultures were incubated for 10 days at 30 °C and 150 rpm in an orbital shaker (Actum, Colombia). Daily samplings were performed to evaluate pH, culture purity and to quantify soluble P. The soluble P in submerged cultures was determined by the phosphomolybdenum blue method, according to the methodology proposed by Murphy and Riley (1962) and modified by Deaker *et al*., (2011), allowing the measurement of the orthophosphate released as product of P solubilization in the culture medium (Spectrophotometer**,** Thermo Bio 5, USA). The calculation was performed using a calibration curve with KH2PO4 at concentrations of 0 to 200 mg/L. A linear regression analysis was performed, and the Equation 2 was obtained to calculate the concentration of soluble, where *y* is the absorbance and *x* is the concentration of soluble P.

$$y=0,0075x+0,0577$$

(Eq. 2)

**Plant growth promotion in *Solanum lycopersicum* L.**

*S. lycopersicum* response to inoculation with *G. diazotrophicus* was evaluated in seedling stage. The preinoculum was prepared in DYGS medium and the inoculum in LGI-P medium (Cavalcante and Döbereiner 1988) as described by Restrepo *et al*., (2017). Seeds of the Torrano® commercial tomato genotype were used, which were arranged in 72 cores. Sterile soil was used as a substrate. The experimental design had the following variables: one tomato genotype, three isolates of *G. diazotrophicus* (two native isolates and an ATCC strain at a dose of 5 mL/L water), one absolute control (without bacteria) and two doses of P (without phosphorus and with H3PO4 [15ppm]), for a total of eight treatments. Each treatment consisted of 72 seeds at planting time (four replicates with 18 seeds each). The trays were kept under semicontrolled conditions at 23 ± 3 °C in a germinator with plastic cover with 380 nm UV filter, Type Agroclear X®.

During the 38-day duration of this stage, data about physiological characteristics of the seedlings (cotyledons height (mm) completely unfolded cotyledons (days) and formation of completely unfolded first, second and third true leaves (days)) were obtained. At the end of the seedling stage, an estimate of physiological variables total fresh mass, aerial and root mass in grams was made according to the scale developed by Bleiholder *et al*., (2001). Finally, the aerial mass / root mass ratio was calculated to assess the proportion of air and root development based on the treatments.

**Statistical analysis**

An analysis of variance of the data (ANOVA) was performed using the General Linear Model procedure (GLM) by means of the SAS version 9,1 statistical package. Further, mean comparison tests were performed through Duncan’s test (P <0,05).

**RESULTS**

**Evaluation of the solubilization of P *in vitro* conditions**

*G. diazotrophicus* native isolates GIBI025 and GIBI029 and type strain ATCC 49037 have the ability to solubilize the three sources of inorganic phosphate (Ca3(PO4)2, AlPO4 and FePO4) present in the NBRIP media both in semiquantitative and quantitative assays.

In semiquantitative assays where the media were supplemented with tricalcium phosphate, all bacteria evaluated peaked solubilization at 168 hours of incubation, whereas in media supplemented with iron phosphate and aluminum the peaks appear at 48 hours for all bacteria. From the isolates, GIBI025 reached the highest solubilization value with a Solubilization Index (SI) of 4,86, followed by GIBI029 with 4,19 and the type strain with a SI of 4,14 in NBRIP + tricalcium phosphate. In NBRIP + aluminum phosphate, the SI value was 1,67 and 1,43 in NBRIP + iron phosphate for all bacteria evaluated (Table 1).

The presence of the pH indicator bromocresol green made possible to observe a shift in color in the media from blue (pH 7,0) to yellow, as a result of acidification. However, in contrast to the reactions occurred in media supplemented with aluminum phosphate and iron phosphate, in which total acidification was observed, only partial acidification was achieved in medium supplemented with tricalcium phosphate, coinciding with the diameters of the solubilization halos. The remaining area became greenish blue, indicating a neuter to basic pH. Phosphate Solubilization (SI) and Acid Production Indices (API) had a similar behavior in the three bacteria in the different culture media (Table 1).

**Table 1.** Solubilization Index (SI) and Acid Production Index (API) 10 days after incubation (240 hours).

|  |  |  |  |
| --- | --- | --- | --- |
| Microorganism | Tricalcium phosphate\*\* | Aluminum phosphate | Iron phosphate |
| SI (mm) \* | API (mm) | SI (mm) | API (mm) | SI (mm) | API (mm) |
| GIBI025 | 4,86a | 4,86a | 1,67 | 15,00 | 1,43 | 12,86 |
| GIBI029 | 4,19b | 4,19b | 1,67 | 15,00 | 1,43 | 12,86 |
| ATCC 49037 | 4,14b | 4,14b | 1,67 | 15,00 | 1,43 | 12,86 |

\*Similar letters did not differ significantly according to the Duncan´s least significant difference (LSD) test with a *p*-value of 0.05.

\*\*These values correspond to the average of three technical replicates.

**Quantification of soluble P (orthophosphate)**

The bacteria evaluated had the ability to solubilize Ca3(PO4)2, AlPO4 and FePO4 in submerged cultures. However, *G diazotrophicus* GIBI025 and GIBI029 reached maximum orthophosphate release faster compared to strain ATCC 49037 in the media studied. In the media supplemented with tricalcium phosphate, isolates GIBI025 and GIBI029 reached maximum production levels of soluble P of 474,47 and 458,27 mg/L at 48 hours of incubation, respectively (Figure 1). ATCC 49037 produced maximum release of P at 72 hours of incubation, with levels of 456,667 mg/L (Figure 1c), which was overdue compared to the other isolates. These values correspond to the average of three technical replica.

|  |
| --- |
| a |
|  |
| b |
|  |
| c |
|  |

**Figure 1.** Dynamics of release of soluble phosphorus on NBRIP supplemented with tricalcium phosphate in submerged culture for 10 days incubation: **a.** GIBI025. **b.** GIBI029. **c.** ATCC49037.

In cultures containing aluminum phosphate, native isolates showed a maximum release of P at 144 hours of incubation with very similar levels (200,667 mg/L for GIBI025 and 209,267 mg/L for GIBI029). The highest value of P released for strain ATCC 49037 was 17,733 mg/L at 192 hours of incubation (Figure 2). With regard to the behavior of the three bacteria evaluated in media supplemented with iron phosphate, it was seen that the native isolates reached higher levels of soluble P in less time (120 hours), with respect to strain ATCC 49037 (144 hours). Concentrations of soluble P of 88,933 mg/L for GIBI025, 104,20 mg/L for GIBI029 and 51,60 mg/L for ATCC 49037 (Figure 3) were obtained. These values correspond to the average of three technical replicates.

|  |
| --- |
| a |
|  |
| b |
|  |
| c |
|  |

**Figure 2.** Release of soluble phosphorus on NBRIP supplemented with aluminum phosphate in submerged culture for 10 days incubation: **a.** GIBI025. **b.** GIBI029. **c.** ATCC49037.

|  |
| --- |
| a |
|  |
| b |
|  |
| c |
|  |

**Figure 3.** Change in release of soluble phosphorus on NBRIP supplemented with iron phosphate in submerged culture for 10 days incubation: **a.** GIBI025. **b.** GIBI029. **c.** ATCC49037.

In the media supplemented with tricalcium phosphate, the behavior of the microorganisms was similar with an initial decrease in pH followed by alkalization. However, the media inoculated with the native isolates showed the lowest values at 24 hours of incubation (pH 4,11 in both cases) and, from this time, a progressive alkalizing occurred to pH 8,0, stabilizing at 144 hours. In contrast, strain ATCC 49037 reached the minimum pH after 48 hours incubation (pH 4,27) and alkalization of the medium appeared at 96 hours, reaching a pH of 8,0 at 216 hours (Figure 1). Lower pH values contrast with the maximum free P, which could be explained by the action of the organic acids released during the cell growth and development (Macias *et al*., 2020). The three bacteria evaluated decreased the pH of the media supplemented with aluminum phosphate and iron phosphate between 0 and 24 hours of incubation and remained at these values until the end of the experiment (pH 2,5 for native isolates and pH 3,3 for strain ATCC 49037). However, the maximum levels of free P were observed between 120-192 hours of incubation.

***S. lycopersicum* culture response to inoculation with *G. diazotrophicus***

Indigenous isolates GIBI 025 and 029 reached statistically similar responses to the control (P), with values between 0,38 and 0,41 g/plant. However, GIBI 025 (without P) showed the best expression, reaching a behavior statistically similar to GIBI 025 and GIBI 029 with addition of P, indicating increased efficiency for isolate GIBI 025 to solubilization of P immobilized in the soil, resulting in the increased development of plants in the total mass (Table 2). Furthermore, isolates GIBI 025 and GIBI 029 with or without addition of P were more efficient in all cases compared to strain ATCC 49037. The type strain showed the lowest plant growth promotion in the absence of P, with values below the absolute treatment (without addition of P), which could be related to the requirements of P by the bacterium for cell metabolism.

**Table 2.** Comparison of means Duncan of variables related to plant growth promotion in seedlings of tomato inoculated with *G. diazotrophicus* in the presence and absence of phosphorus fertilizer.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables evaluated | GIBI025 | GIBI029 | ATCC 49037 | CONTROL |
| Without P | With P | Without P | With P | Without P | With P | Without P | With P\* |
| Emergence of cotyledons (days)  | 12,06c | 12,22bc | 13,64b | 12,31bc | 13,78b | 13,25bc | 17,47a | 12,47bc |
| First true leaf (days)  | 22,75c | 22,53c | 23,97bc | 22,47c | 24,92b | 23,08c | 29,42a | 22,72c |
| Second true leaf (days) | 23,58c | 23,78c | 25,39c | 23,47c | 27,36b | 24,33c | 31,19a | 23,69c |
| Third true leaf (days)  | 32,26c | 31,89cd | 34,16b | 31,64cd | 34,52b | 31,53cd | 35,81a | 31,31d |
| Height to cotyledons day 20 (mm) | 25,42a | 25,25a | 20,94b | 25,14a | 22,31ab | 23,81ab | 14,78c | 24,78a |
| Height to cotyledons day 22 (mm) | 29,97a | 28,19ab | 26,53b | 27,69ab | 25,78b | 27,31ab | 19,36c | 27,97ab |
| Height to cotyledons day 24 (mm) | 32,17a | 29,72b | 28,36b | 29,22b | 27,50b | 29,03b | 22,86c | 29,50b |
| Height to cotyledons day 38 (mm) | 33,88a | 30,92b | 30,31bc | 30,08bc | 29,80c | 30,75bc | 29,22d | 30,56bc |
| Total fresh mass (g) | 0,33b | 0,38ab | 0,22c | 0,39ab | 0,06d | 0,25c | 0,12d | 0,41a |
| Aerial fresh mass (g) | 0,31a | 0,34a | 0,20b | 0,35a | 0,05d | 0,23b | 0,11c | 0,36a |
| Root fresh mass (g) | 0,02b | 0,04a | 0,02b | 0,04a | 0,01c | 0,02b | 0,01c | 0,05a |
| Root mass / air mass ratio | 0,06 | 0,12 | 0,10 | 0,11 | 0,20 | 0,09 | 0,09 | 0,14 |

\*Similar letters did not differ significantly according to the Duncan´s least significant difference (LSD) test with a *p*-value of 0,05.

The maximum values of SI of the three bacteria in NBRIP + aluminum phosphate and NBRIP + iron phosphate were similar in the semiquantitative assays. Statistically significant differences were found in submerged cultures between the indigenous isolates and the type strain.

The protocol usually employed for the selection of phosphate solubilizing microorganisms involves the growth of microorganisms in solid medium, using insoluble inorganic phosphate as the sole source of available P, usually Ca3(PO4)2. A halo is formed as a result of cell growth and metabolism, with a size proportional to the intensity of solubilization (Widdig *et al*., 2019). One disadvantage of this method has to do with the fact that, in the soil, the fraction of inorganic P available interacts with different metal elements according to the pH, producing complexes with aluminum and iron in acidic soils (FePO4 or AlPO4) and with calcium in alkaline soils (Ca3(PO4)2) (Penn and Camberato, 2019). For this reason, in this work it was decided to use three different forms of insoluble P (FePO4, AlPO4 and Ca3(PO4)2) as sources of phosphate for the initial evaluation of the phosphate solubilizing ability of the bacteria under study.

Numerous authors have found that the semiquantitative method based on screening plate often presents conflicting results by subjecting microorganisms to assessment in their ability to solubilize P in liquid media (Ibáñez *et al*., 2021; Bashan *et al.*, 2013). This may be due to how microorganisms interact with P complexes in the different matrices: in solid media, the growth area of the colony limits the contact of microorganisms with P complexes, which is confined to the small space under and around the colony. On the other hand, degradation of complexes in liquid media can be more efficient due to the adherence and attack of many microorganisms in the different dimensions of the pellets, leading to a more efficient solubilization. Due to the abovementioned, it is considered that the quantification of free P in submerged culture provides a more objective measurement of the ability of microorganisms to degrade insoluble P.

In quantification of soluble P late increases occurred in the release of P between 192 and 216 hours of incubation in media supplemented with aluminum phosphate and iron phosphate. Such increases could be related to the stage of decline or death of the bacteria, where the P incorporated into the biomass is subsequently released as a result of cell lysis (Restrepo *et al.*, 2015).

Inorganic phosphate solubilization is often associated with decreasing pH in the culture medium, which is often associated with the production of organic acids such as gluconic, keto-gluconic and lactic. This decrease is not strictly proportional to the amount of P released (Macias *et al*., 2020). Some studies have found a direct correlation between decreasing pH and increasing soluble P in crops, but other reports have not observed this phenomenon (Penn and Camberato, 2019). This suggests that, besides the production of acid, other mechanisms are involved in microbial solubilization of the various sources of phosphate. In this sense, Estrada *et al*., (2013) found that the maximum value of P solubilized by *G. diazotrophicus* ATCC 49037 was 239 mg/L in NBRIP supplemented with tricalcium phosphate, reporting a direct relationship between the solubilization of P and the decrease of pH.

The root mass / aerial mass ratio showed its best behavior in the control treatments with P and bacteria with addition of P. However, despite this relationship being halved in the case of isolate GIBI025, a change is evident only in fresh root mass, which is reduced by half, preserving the value of the aerial mass, while in isolation GIBI029, both the aerial part and the root portion are reduced.

Previous studies (Restrepo *et al.*, 2015) showed AIA values for isolates GIBI 025, 029 and strain ATCC of 17,47, 78,54 and 64,28 µg/mL, respectively. These values, relative to the total mass of the plant, indicate that the lowest value reported of AIA reached by isolate GIBI025 is sufficient to promote growth of tomato seedlings, achieving the best values in the absence of P (0,33 g / seedling). Sánchez *et al*., (2014) reported values between 18 and 25 µg/mL of total indole compounds, indicating that these levels may be considered as plant growth promoters. It is considered that high concentrations can inhibit the growth of seedlings, which may have occurred with bacteria GIBI029 and ATCC 49037, which showed the highest levels described above.

In the case of development variables associated with growth for variables appearance of cotyledons, first, second and third entirely deployed true leaves, it was found that the absence of P in all cases causes a lengthening of the period in days for the organ to develop, with differences between 5 and 7 days in the absence of P. Isolate GIBI025 showed the best behavior reaching the shortest time in the absence of P, with no statistical differences (P <0,05) compared to the treatments with addition of P, reaching a shortening in the period of development in each stage between 5 and 7 days, equivalent to a reduction between 16 and 20% of the time in the stage of development of the tomato seedling in the conditions of the evaluation (38 days). The trend in development for variable height to cotyledons over time (20 to 38 dap) remains as in the previous case. The best values are reached in treatments with the presence of P in contrast to treatments without addition of this element, except the case of isolate GIBI 025, which retains the positive effect even in the absence of P, evidencing the promising activity of this isolate as plant growth promoter.

Yadav *et al*., (2021) described the importance of nutrients in plants as and their feature as essential elements because they cannot be replaced by others in function and their absence generates a delay in the normal development of the plant. This explains the behavior of the absolute control in contrast to the other treatments; it is also an indication of microbial activity and the ability to mobilize and solubilize soil phosphate, which resulted in the differential development of seedlings in the treatments with different isolates in the absence of fertilizer. It is important to highlight the fact that the phosphorus fertilizers applied in this study were highly soluble, making them bioavailable, and plant roots can capture them immediately. The process of microbial solubilization of P is a phenomenon that depends on various microbial metabolic mechanisms, and the release of the metabolites involved takes some time to take place. Despite this, isolate GIBI025 appears to be a bacterium with greater efficiency and speed to solubilize the P. However, other experiments should be developed to test this hypothesis.

It was also noted that all isolates and the type strain improved the height reached by the cotyledons on days 20, 22, 24 and 38, reaching significant differences with respect to the absolute control. Isolate GIBI025 caused a statistical difference comparable or even superior in the abovementioned variables versus the agronomic control (Table 2). This could be demonstrating synergistic activity of Plant Growth Promoting Bacteria both as phosphate solubilizing and in the production of growth regulators such as auxins, gibberellins and cytokinins - which have an effect on the elongation of the meristem tissues of plants – since, if the results were similar to the agronomic control, this would evidence only an effect of the bacterium as P solubilizing.

Only native isolates GIBI025 and GIBI029 significantly increased the fresh weight of the different parts of the seedling. It is worth mentioning that isolate GIBI025 significantly improved the different variables related to plant growth promotion evaluated similarly or even higher than when the bacterium was used in the company of phosphorus fertilizer (Table 2).

Different studies have reported that phosphate solubilizing microorganisms can improve the growth and production of different crops of economic interest. Blanco-Vargas *et al*., (2020) isolated *Pseudomonas* sp., (A18) and *Serratia* sp., (C7) from soils at the “*Departamento de Boyacá*” Colombia, where *Allium cepa* is cultivated. Bacteria were cultured in MT11B media and evaluated as a bio-fertilizer for *A. cepa* germination and growth during two months at greenhouse scale. In MT11B media growth curve (12 h) demonstrated that co-culture can grow in the presence of PR (as a inorganic source of phosphorus) and glucose concentrations 7,5-fold, lower than in SMRS1 media and brewer's yeast hydrolysate; producing phosphatase enzymes with a volumetric activity of 1,3 ± 0,03 PU at 6 h of culture and 0,8 ± 0,04 PU at 12 h. Moreover, co-culture released soluble phosphorus at a rate of 58,1 ± 0,28 mg L−1 at 8 h and 88,1 ± 0.32 mg L−1 at 12 h.  Elhaissoufi *et al*., (2020) investigated above- and below-ground responses in wheat fertilized with rock P (RP) under controlled conditions of five contrasting PSB (*Pseudomonas* spp.) isolates (low “PSB1”, moderate “PSB2 and PSB4” and high “PSB3 and PSB5” P-solubilizing capacity “PSC”). All PSB isolates increased wheat root traits, particularly PSB5 which increased root biomass and PSB3 that had greater effect on root diameter in 7-, 15- and 42-day old plants. Romero-Perdomo *et al*., (2021) evaluated six plant growth-promoting bacteria strains under greenhouse conditions. The *Rhizobium* strain B02 significantly promoted growth, shoot P content and photosynthetic rate. Application of *Rhizobium* strain B02 showed the capacity to optimize the use of low-solubility fertilizer as the rock phosphate.

This study used the measurement of physiological variables related to plant growth promotion as an indirect measurement of phosphate solubilization after inoculation with different isolates of *G. diazotrophicus*. However, these parameters may be the result of the combined action of different mechanisms of action present in this bacterium, such as biological nitrogen fixation, production of indoleacetic acid and other phytohormones. An important part of the improvement of variables growth and development evaluated in *S. lycopersicum* is due to the mobilization of phosphate mediated by *G. diazotrophicus*. This is supported by the fact that the treatments with microorganisms, in the absence of phosphorus fertilization, produced comparable or superior results to the control with chemical fertilizer.

However, it is important to consider in this crop if chemical fertilization (phosphoric rock) can improve its effectiveness with the use of phosphate-solubilizing bacteria, a condition that was demonstrated in *Medicago trunculata* (Ben *et al*., 2020).

**CONCLUSIONS**

The microorganisms that are naturally in the environment can interact with the different fractions of insoluble P and turn them into soluble forms within the soil solution, from where it can be absorbed by plant roots. The use of these microorganisms could reduce the use of phosphate fertilizers and is an approach that contributes to the establishment of cleaner and more sustainable agricultural practices. The indigenous isolates of *G. diazotrophicus* from Colombian agroecosystems are potentially efficient in solubilizing various sources of inorganic phosphate and promoting plant growth in *S. lycopersicum* L. at seedling level, which is backed by the increases in plant growth variables.

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