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Endophytic bacteria promote growth and increase the aloin content of *Aloe vera*

[Las bacterias endofíticas promueven el crecimiento y aumentan el contenido de aloína del *Aloe vera*]

Cintia Faria da Silva[1,2], Luciana Cristina Vitorino[2], Isabelle Guimarães de Oliveira[2], Thales Caetano de Oliveira[2], Erika Crispim Resende[3], Paulo Sérgio Pereira[2] & Edson Luiz Souchie[2]

[1]Postgraduate in Biotechnology and Biodiversity, Universidade Federal de Goiás, Goiânia, Brazil
[2]Laboratory of Agricultural Microbiology, Instituto Federal Goiano, Rio Verde campus, Rio Verde, Brazil
[3]Department of Biomolecules, Instituto Federal Goiano, Iporá campus, Iporá, Brazil.

Reviewed by:
María Elena Cazar
Universidad de Cuenca
Ecuador

Patricia Peralta
Instituto Nacional de Tecnología Agropecuaria
Argentina

Correspondence:
Luciana Cristina VITORINO:
luciana.vitorino@ifgoiano.edu.br

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Abstract: *Aloe vera* is among the world's economically most important medicinal plants, but as the growth of this plant and, consequently, the accumulation of metabolites is slow, we tested the hypothesis that root endophytic bacteria isolated from *A. vera* plants can promote growth and increase the accumulation of aloin in the gel and latex. For this, we inoculate seedlings with four endophytic bacteria and a combination of them. We confirmed the hypothesis and identified two strains with potential for the formulation of inoculants to improve the cultivation of *A. vera*. The bacterium 149H *Paraburkholderia* sp. increases the number of leaves and the accumulation of biomass, but on the other hand, 35V *Enterobacter ludwigii* inoculation increased the content of aloin in the gel and in the latex. Further research should focus on the association of these two strains in a single inoculant, to both promote growth and increase the synthesis of metabolites.

Keywords: Medicinal plants; Inoculants; Endophytic bacteria; Plant growth promotion; PGPR

Resumen: *Aloe vera* se encuentra entre las plantas medicinales económicamente más importantes del mundo, pero como el crecimiento de esta planta y, en consecuencia, la acumulación de metabolitos es lento, probamos la hipótesis de que las bacterias endofíticas de raíces aisladas de las plantas de *A. vera* pueden promover el crecimiento y aumentar la acumulación de aloína en el gel y látex. Para ello, inoculamos plántulas con cuatro bacterias endofíticas y una combinación de ellas. Confirmamos la hipótesis e identificamos dos cepas con potencial para la formulación de inoculantes para mejorar el cultivo de *A. vera*. La bacteria 149H *Paraburkholderia* sp. aumenta el número de hojas y la acumulación de biomasa, pero, por otro lado, la inoculación con *Enterobacter ludwigii* 35V aumentó el contenido de aloína en el gel y en el látex. La investigación adicional debe centrarse en la asociación de estas dos cepas en un solo inoculante, tanto para promover el crecimiento como para aumentar la síntesis de metabolitos.

Palabras clave: Plantas medicinales; Inoculantes; Bacterias endofíticas; Promoción del crecimiento vegetal; PGPR

INTRODUCTION

Aloe vera (L.) Burm f. is a species of the family Xanthorrhoeaceae (Amoo *et al*., 2014) and is not only among the world's most economically important medicinal plants, but also has enormous biocultural value (Grace, 2011). The plants of the genus *Aloe* have CAM (Crassulacean Acid Metabolism) photosynthesis, which minimizes water loss, as well as being capable of storing water in their leaves, which allows them to survive droughts (Cousins & Witkowski, 2012). *Aloe* leaves have a waxy surface, which reflects excess sunlight and minimizes evaporation through the stomata and external cells (Oda & Erena, 2017). The leaf has three layers – gel, latex, and cuticle. The gel is the most internal layer, and is formed by soft, mucilaginous tissue, which is transparent and lubricous, with large parenchymal cells, containing 99% water. It is composed of glucomannans, amino acids, lipids, sterols, and vitamins (Surjushe *et al*., 2008). The latex is an intermediate layer, with a bitter yellow sap that contains anthraquinones and glycosides (Hamman, 2008). The cuticle is the thick outer layer, which forms a protective skin, in which carbohydrates and proteins are synthesized (Maan *et al*., 2018). The root system grows only a few centimeters below the surface of the soil, and absorbs water efficiently, even in areas with low precipitation (Cousins & Witkowski, 2012).

The traditional ethnomedicinal use of *A. vera*, together with recent pharmacological and phytochemical research, which has investigated the plant's many active compounds, such as amino acids, sugars, enzymes, vitamins, minerals, saponins, anthraquinones, lignin, and salicylic acid (Misir *et al*., 2014), indicate that the plant is beneficial for the treatment of a range of diseases. The chemical composition of *A. vera* is extremely diverse, and the plant is rich in secondary metabolites, which are mostly associated with the gel found inside the leaves, with more than 75 known biological properties (Radha & Laxmipriya, 2015; Bjørklund *et al*., 2018; Mendy *et al*., 2019; Añibarro-Ortega *et al*., 2019; Sonawane *et al*., 2020). The most important compounds are aloin (barbaloin), emodin, isobarbaloin, aloe-emodin, aloesin, aloeresin, isoaloeresin, and acemannan (Hamman, 2008; Surjushe *et al*., 2008; Domínguez-Fernández *et al*., 2012; Baruah *et al*., 2016; Salah *et al*., 2017).

According to the International Aloe Science Council, products derived from the leaves of *A. vera* support a global market of US $13 billion, which attracts the interest of investors worldwide (Schulz, 2012). *Aloe vera* is planted widely in warm, dry regions (Hazrati *et al*., 2017), where it grows best on well-drained soils (Cristiano *et al*., 2016). However, this plant grows extremely slowly, which drives the demand for the development of techniques that promote the accumulation of biomass by *A. vera* plants and, in particular, that stimulate the production of the key metabolites that accumulate in the gel and latex of this species.

Plants can select different bacterial communities from the soil through the production of root exudates. Promising results in the promotion of plant growth, such as increased biomass, and increased stem size and root depth, have been obtained by inoculating plants or seeds with endophytic bacteria, including *Achromobacter*, *Microbacterium*, *Bacillus* (Soares *et al*., 2016), *Enterobacter* (Khalifa *et al*., 2016; Castro *et al*., 2018; Ludueña *et al*., 2018), *Pantoea* (Khamwan *et al*., 2018; Marag & Suman, 2018), *Paraburkholderia* (Bernabeu *et al*., 2018), *Pseudomonas* (Ali *et al*., 2014), *Lactococcus*, and *Klebsiella* (Marag & Suman, 2018). Given this, we tested the hypothesis that the artificial inoculation of endophytic bacteria previously isolated from the roots of *A. vera* induce growth and stimulate the synthesis of aloin in these plants. In this context, bio-inputs, such as bacterial strains and strain mixtures, if they demonstrate positive effects on growth, can increase productivity and add value to the production of *A. vera*. This effect was recently suggested by Khajeeyan *et al*. (2021).

Despite the growing interest in the bioactive properties of *A. vera* (Bajpai, 2018; Gao *et al*., 2018a), few published data are available on the influence of symbiotic microorganisms on the growth and metabolism (Gupta *et al*., 2012; Sharma *et al*., 2014) of this species. We thus decided to investigate the potential of root endophytic bacteria, isolated from *A. vera* plants grown in soils of the Cerrado biome, to induce positive effects on biometric parameters, as well as the aloin content in the gel and latex, in an experiment conducted in the vegetation. Our ultimate objective was to identify bacterial strains with good potential for bioinoculation in the cultivation of *A. vera*.

MATERIAL AND METHODS

Selection of bacterial strains

Thirty-four bacterial isolates were obtained previously from the roots of *Aloe vera* growing in three distinct environments (Silva *et al*., 2020): (1) a nursery, in diverse vegetation, including both arboreal and herbaceous species; (2) a garden, in contact with herbaceous plants, mainly medicinal species, subject to periodic gardening, and (3) a farm field, in a anthropogenic area with little vegetation. The importance of these isolates was assessed based on their different functional traits: the production (μg mL^{-1}) of indole-3-acetic acid (IAA), solubilization (mg mL^{-1}) of natural phosphate from Bayóvar, Peru (12.8% P), tricalcium phosphate ($Ca_3(PO_4)_2$) and iron phosphate ($FePO_4$) (mg mL^{-1}), and antibiosis (relative inhibition – RI %) against the phytopathogenic fungus *Sclerotinia sclerotiorum*, the phytopathogen *Fusarium* sp., and *Rhizoctonia* sp.

These functional data were evaluated together in a correlation matrix and combined in a Principal Components Analysis (PCA). As the variables were measured in different units, a correlative PCA was applied, with the data being standardized to have a mean of 0 and standard deviation of 1. The number of components was selected according to the eigenvalues (>1.0) and the explained variance (>80%). The variables were also evaluated using Pearson's correlation coefficient, with the strength of the relationship being evaluated by the *r* value, and the significance of the interaction being tested using a 5% probability threshold. These statistical analyses were run in the R environment, version 3.4.3. (R Core Team, 2021).

The behavior of the different isolates in relation to the variables in the PCA, as well as the results of the correlation, were used for the selection of the isolates to be tested as growth promoters and stimulators of the production of aloin in *A. vera.* This selection included at least one strain from each environment. Thus, strains 35V (*Enterobacter ludwigii*) and 135V (*Enterobacter tabaci*) were selected from the nursery, 149H (*Paraburkholderia* sp.) from the garden, and 389C (*Pantoea cypripedii*) from the field.

Preparation of the inoculants

Isolates 35V, 135V, 149H, and 389C were cultivated separately in nutrient broth at ambient temperature in a table-type agitator (150 rpm), for 24 h. The final concentration of the cultures was standardized to 10^9 CFU mL^{-1} using autoclaved distilled water. Finally, 100 mL of the different standardized cultures were prepared. The preparation of the consortium was based on a combination of all four cultures, of equal cellular density, mixed in an Erlenmeyer, with 20 mL of the standardized cultures of each isolate being mixed. The *A. vera* seedlings were inoculated or not (control) with either one strain or the consortium of the four strains.

Growth promotion experiment

The experiment was conducted between September 2017 and May 2018, in 6 kg plastic pots containing a sterilized, sieved soil:sand (2:1) substrate, which was dried for 12 days. This soil consisted of samples of red dystroferric latosol collected from an area of native Cerrado (17°48'28" S, 50º53'57" W, 720 masl), at a depth of 10–40 cm. The chemical composition of this soil was pH in $CaCl_2$ = 4.4; P = 1.1 mg dm^{-3}; K = 76.7 mg dm^{-3}; Ca = 0.47 $cmol_c$ dm^{-3}; organic matter = 31.8 $g{\cdot}kg^{-1}$, and base saturation = 11%. The pots were filled with approximately 4 kg of this substrate. The *A. vera* seedlings, with 3–5 leaves and a height of 10–15 cm were removed carefully from the three matrices and transplanted to the pots, which were initially keep in a greenhouse for 40 days, for adaptation. After 40 days, the plants were inoculated with 5 mL of the bacteria (10^9 CFU mL^{-1}) at the base of the plants, which were then kept in a greenhouse before being transferred to the open-air environment. The experiment had a fully randomized design, with six treatments: control; 35V - *Enterobacter ludwigii*; 135V - *Enterobacter tabaci*; 149H - *Paraburkholderia* sp.; 389C - *Pantoea cypripedii*; MIX - mixture of all four bacteria, with 10 repetitions. The plants were irrigated twice a week. During the experiment, the fungicide Carbendazim (2 mL L^{-1}) was applied five times for the control of fungal disease in the aerial part of the plant.

Biometric evaluation of plant growth promotion

Six months after inoculation, the plants were removed carefully from the pots to determine their biometric parameters: seedling length (cm), the number of leaves, fresh and dry biomass of the leaves and roots (g), root volume (mm^3), and root diameter (mm). The seedling length was based on the length of the largest leaf observed and the fresh and dry

biomass was obtained by weighing the two largest leaves, as the remaining leaves were used for the extraction of the gel and latex, which was necessary to determine the aloin content. The root volume and diameter were obtained using the SAFIRA software (Jorge & Silva, 2010). As the roots of *A. vera* are fasciculated, the diameter was obtained by averaging the diameter of all the roots.

Determination of the aloin content

The *A. vera* leaves were washed under running water and then rinsed with distilled water. The leaves were then cut at the base to drain off the latex, which was collected over the subsequent two hours. The gel (parenchymal tissue) was separated mechanically. The samples were wrapped in aluminium foil before being stored in flasks to impede the decomposition of the aloin. They were then frozen and lyophilized.

The samples of the latex and gel were analyzed individually to quantify the concentration of aloin. The extracts were obtained from 60 mg of latex and 100 mg of the lyophilized gel, in 5 mL of methanol. All the samples were sonicated for 10 minutes and filtered through cottonwool and a Whatman PP 0.45 µm nylon membrane prior to the chromatographic analysis.

A high efficiency Shimadzu® liquid chromatograph, equipped with an LC-20AT quaternary pump system, CTO-20A column oven, SIL-20AHT auto-injector, and DAD SPD-M20A detector, was used to analyze the samples. The samples were injected into a Shimadzu® C_{18} Shim Pack VP-ODS (150 mm x 4.6 mm; 5 µm) chromatographic column coupled to a Shimadzu® ODS pre-column (10 mm x 4.6 mm; 5.0 µm). The chromatographic data were analyzed in the LC-Solution® program.

The chromatographic conditions were as follows: mobile phase A (ultrapure water + 0.1% of acetic acid); mobile phase B (acetonitrile), gradient mode: (Min/A%:B%): 0/80:20; 11.5/50:50; 13/20:80; 19/80:20, with an oven temperature of 40°C; mobile phase flow of 1.0 $mL.min^{-1}$, detection at 254 nm, and injection volume of 20 µL. The latex samples were diluted (1:10) for injection, while the gel samples were injected without previous dilution.

The linearity of the method was verified based on the calibration curve. For this, five different solutions were prepared with different concentrations of aloin (standard aloin, from *Aloe barbadensis* Miller (syn *Aloe vera* (L.) Burm. f.) leaves, ≥ 97% Sigma-Aldrich, CAS 1415-73-2, $C_{21}H_{22}O_9$) (25; 50; 125; 250 and 500 µg mL^{-1} in methanol from a stock solution of 2.5 mg mL^{-1}). The aloin was identified comparing the retention time with that of the standard compound. The quantity of aloin produced by the plant was calculated based on the sample peak, concentration, and the area of the standard peak, in five repetitions per treatment.

The retention time of the aloin was 9.17 min (Figure No. 1), which was identified by comparison with the standard value and by using characteristic spectra, obtained with a DAD detector. The method was linear between 25 and 500 µg mL^{-1} for the aloin content present in the latex and gel samples. The linearity was expressed by the coefficient of determination (r = 0.997) and the equation obtained by the analysis was y=13536x – 35310. The relative standard deviations between the injections and the standard aloin were less than 5% (between 0.37 and 0.68%).

Statistical analysis

The normality of the data was verified using the Shapiro-Wilk test. As the assumption of normality was satisfied, an Analysis of Variance (ANOVA) was applied. The biometric means and the aloin content, observed in the treatments were compared with the control using Dunnett's test run in the GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Selection of bacterial strains

The 149H strain of *Paraburkholderia* sp. was selected for having the highest means for the solubilization of Bayóvar phosphate (45 mg mL^{-1}), the highest IRs against *Fusarium* sp. (32.2%), and for synthesizing large amounts of IAA (132.6 μg mL^{-1}). The PCA indicated a tendency of this bacterium to excel in relation to these variables (Figure No. 1a). The 389C *Pantoea cypripedii* isolate was chosen to test its ability to promote growth in *A. vera* because it excelled in the solubilization of $Ca_3(PO_4)_2$ and $FePO_4$. This bacterium solubilized the largest amounts of these phosphates (36.5 mg mL^{-1} and 2.8 mg mL^{-1}, respectively). The 135V *Enterobacter tabaci* isolate was selected for its high capacity for IAA synthesis (225.2 μg mL^{-1}) and also for being moderately associated with solubilization of Bayóvar

phosphate (13.0 mg mL^{-1}), while the 35V *Enterobacter ludwigii* isolate was chosen for being associated moderately with most of the functional traits evaluated, that is, IAA synthesis (122.8 μg mL^{-1}), Bayóvar phosphate solubilization (12.3 mg mL^{-1}), $Ca_3(PO_4)_2$ solubilization (12.6 mg mL^{-1}), and antibiosis against *S. sclerotiorum* (31.4 %).

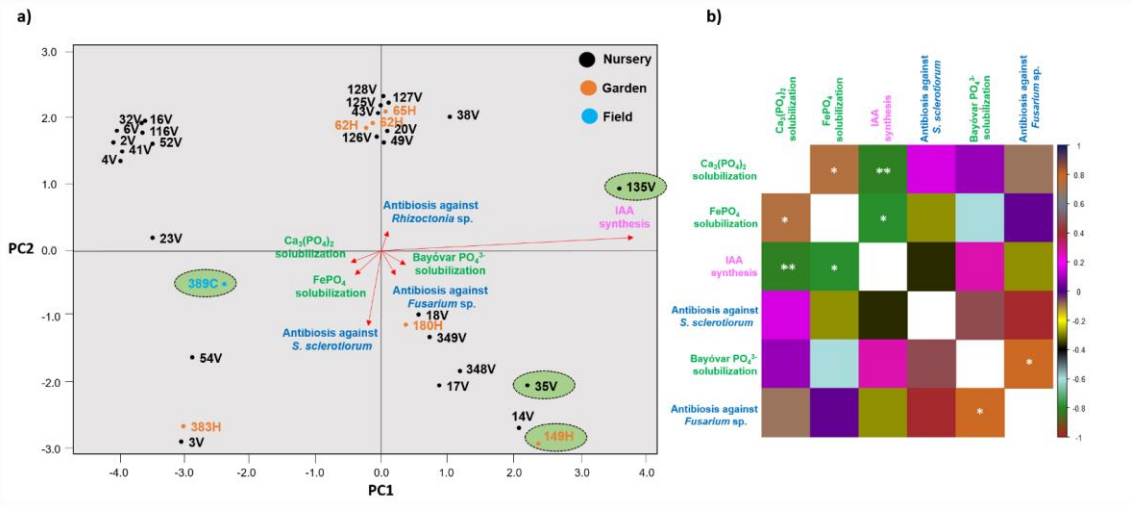

Figure No. 1

(a) Correlative principal component analysis for the functional trait data on 34 root endophytic bacteria, isolated from *Aloe vera* and (b) the Pearson correlation matrix obtained for the same data. 389C = *Pantoea cypripedii*, 62H = *Enterobacter* sp., 63H = *Enterobacter* sp., 65H = *Enterobacter* sp., 149H = *Paraburkholderia* sp., 180H = *Enterobacter* sp., 383H = *Lysinibacillus macroides*, 2V = *Bacillus megaterium*, 3V = *Brevibacillus agri*, 4V = *Bacillus megaterium*, 6V = *Lysinibacillus xylanilyticus*, 14V = *Enterobacter tabaci*, 16V = *Lysinibacillus macroides*, 17V = *Enterobacter tabaci*, 18V = *Enterobacter tabaci*, 20V = *Enterobacter tabaci*, 23V = *Microbacterium aerolatum*, 32V = *Chryseobacterium taiwanense*, 35V = *Enterobacter ludwigii*, 38V = *Enterobacter asburiae*, 41V = *Bacillus megaterium*, 43V = *Enterobacter tabaci*, 49V = *Enterobacter ludwigii*, 54V = *Pantoea agglomerans*, 116V = *Chryseobacterium taiwanense*, 125V = *Chryseobacterium taiwanense*, 126V = *Enterobacter ludwigii*, 127V = *Enterobacter ludwigii*, 128V = *Enterobacter tabaci*, 135V = *Enterobacter tabaci*, 348V = *Enterobacter tabaci*, 349V = *Lelliottia nimipressuralis*. *significant at a probability of 5%, **significant at 1%

None of the selected isolates inhibited the growth of *Rhizoctonia* sp., however. When the means recorded for the other functional traits were correlated, a significant negative relationship was found between the synthesis of IAA and the solubilization of both $Ca_3(PO_4)_2$ (r = -0.814, p>0.001) and $FePO_4$ (r = -0.787, p=0.021), which supported the choice of the 135V *Enterobacter tabaci* strain as an IAA producer and the 389C *Pantoea cypripedii* strain as a solubilizer of $Ca_3(PO_4)_2$ and $FePO_4$. The relationship between the solubilization of Bayóvar phosphate and antibiosis against *Fusarium* sp. was also significant (r = 0.780, p=0.036), supporting the selection of 149H *Paraburkholderia* sp. to represent these two functional traits.

Biometric analyses

Treatments with endophytic bacteria that promote plant growth, applied individually as inoculants, did not affect the growth of the *A. vera* seedlings, with growth being similar to that of control in all the seedlings of the different bacterial treatments (Figure No. 2a). However, the plants inoculated with 149H *Paraburkholderia* sp. did develop a significantly larger number of leaves (10.75 ± 0.63) than the control plants (8.50 ± 0.50), that is, an increase of

25.9% in this parameter related to the application of this isolate (Figure No. 2b).

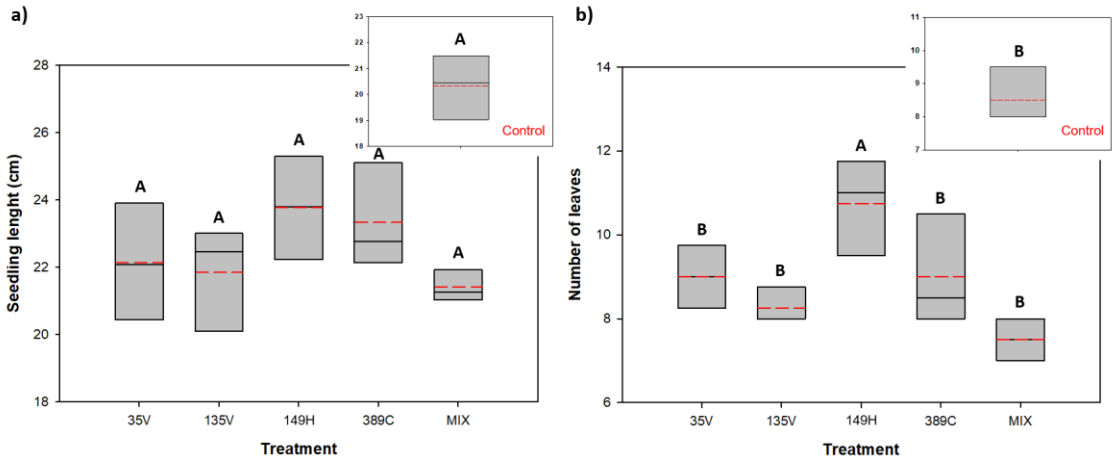

Figure No. 2
(a) seedling length (cm) and (b) the number of leaves observed in the *Aloe vera* seedlings inoculated with different strains of growth-promoting endophytic bacteria isolated from *A. vera* roots. Control = not inoculated; 35V = *Enterobacter ludwigii*; 135V = *Enterobacter tabaci*; 149H = *Paraburkholderia* sp.; 389C = *Pantoea cypripedii*; MIX = consortium of all four bacteria. The boxes represent the maximum and minimum values observed for each treatment. The black line represents the median and the red line, the mean. Different upper case letters above the columns indicate a significant difference between the means

The 149H *Paraburkholderia* sp. isolate also had a significant effect on the mean fresh biomass of the leaves (59.24 ± 6.59 g) and roots (23.64 ± 5.22 g), with the mean leaf biomass being 51.8% heavier than that of the control plants (39.05 ± 2.48 g; Figure No. 3a), and the roots 107% heavier than the control (11.37 ± 0.64 g; Figure No. 3b). None of the treatments did not affect the dry weight of the leaves significantly, however (Figure No. 3c). The plants treated with 149H *Paraburkholderia* sp. had the greatest dry leaf biomass (3.41 ± 0.25 g), but this did not differ significantly from the mean mass in the control plants (3.34 ± 0.10 g). This same isolate nevertheless had a significant effect on the dry weight of the root (Figure No. 3d), with the mean weight in this treatment (1.70 ± 0.74 g) being 11.17% greater than the control (1.51 ± 0.03 g).

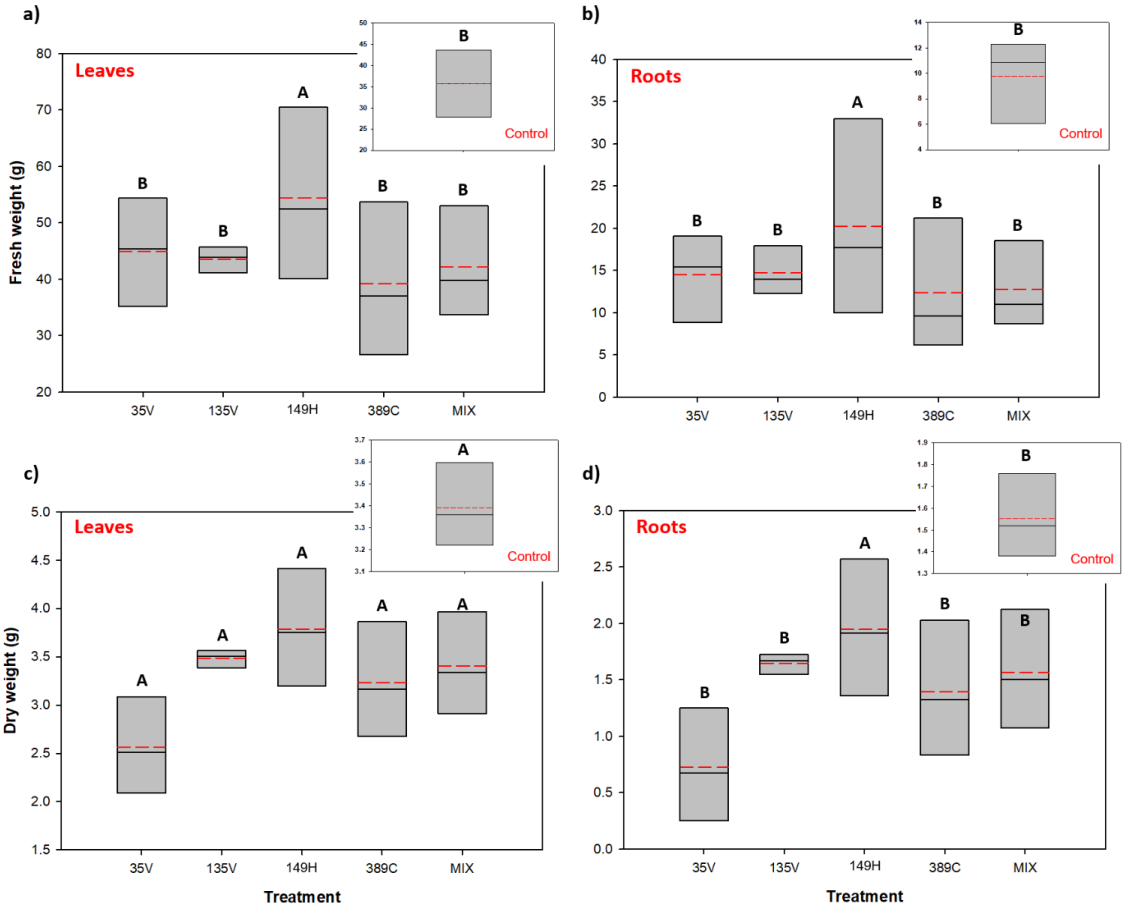

Figure No. 3
Fresh weight in grams of the (a) leaves and (b) roots, and the dry weight in grams of the (c) leaves and (d) roots of the *Aloe vera* seedlings inoculated with the different strains of growth-promoting endophytic bacteria isolated from *A. vera* roots. Control = not inoculated; 35V = *Enterobacter ludwigii*; 135V = *Enterobacter tabaci*; 149H = *Paraburkholderia* sp.; 389C = *Pantoea cypripedii*; MIX = consortium of all four bacteria. The boxes represent the maximum and minimum values observed for each treatment. The black line represents the median and the red line, the mean. Different upper case letters above the columns indicate a significant difference between the means

Inoculation with 135V *Enterobacter tabaci* promoted a significant reduction in root volume (Figure No. 4a), with the plants inoculated with this bacterium having a much low mean for this parameter (16.59 ± 2.44 mm^3) compared with the control plants (37.41 ± 0.09 mm^3). The other isolates did not have a negative effect on root volume, however (Figure No. 4a). The root surface area (Figure No. 4b) was affected negatively by 35V *Enterobacter ludwigii*, 135V *Enterobacter tabaci*, and the consortium of isolates (MIX), which all had significantly lower mean values (37.82 ± 3.83; 32.77 ± 0.93; 27.98 ± 2.13 mm^2, respectively) in comparison with the control plants (58.00 ± 2.09 mm^2). By contrast, 35V *Enterobacter ludwigii* was the only treatment to have a significantly positive effect on root diameter (Figure No. 4c), with a mean of 0.81 ± 0.06 mm, which represents an increase of 33.3% over the control (0.61 ± 0.01 mm).

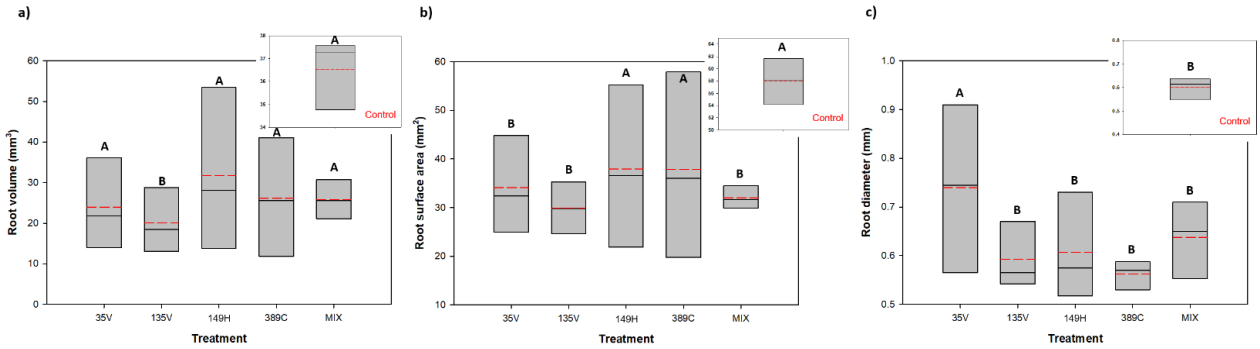

Figure No. 4

The (a) volume (mm^3), (b) surface area (mm^2), and (c) diameter (mm) of the roots of the *Aloe vera* seedlings inoculated with different strains of growth-promoting endophytic bacteria isolated from *A. vera* roots. Control = not inoculated; 35V = *Enterobacter ludwigii*; 135V = *Enterobacter tabaci*; 149H = *Paraburkholderia* sp.; 389C = *Pantoea cypripedii*; MIX = consortium of all four bacteria. The boxes represent the maximum and minimum values observed for each treatment. The black line represents the median and the red line, the mean. Different upper case letters above the columns indicate a significant difference between the means

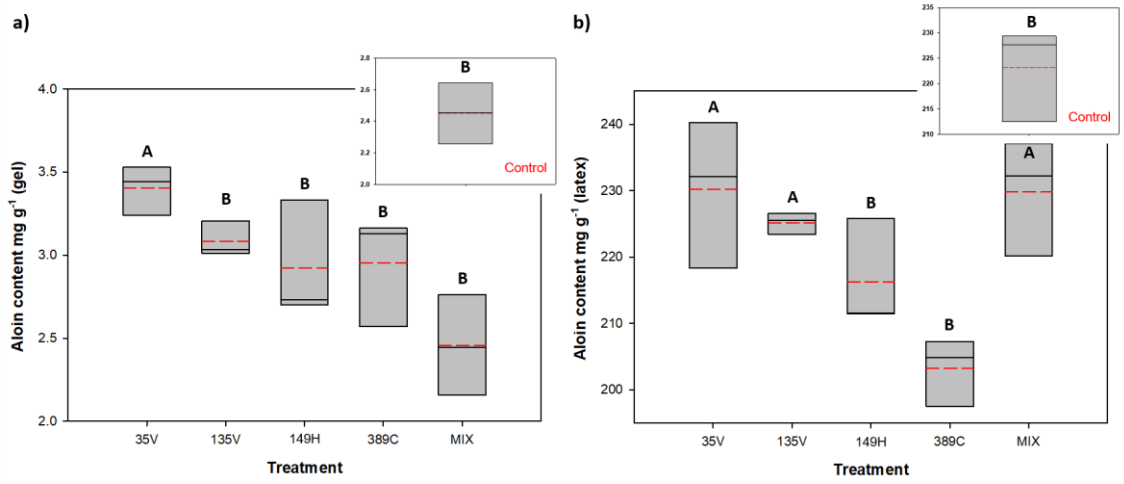

Figure No. 5

Aloin content (mg g^{-1}) in the (a) gel and (b) latex of the *Aloe vera* seedlings inoculated with different strains of growth-promoting endophytic bacteria isolated from *A. vera* roots. Control = not inoculated; 35V = *Enterobacter ludwigii*; 135V = *Enterobacter tabaci*; 149H = *Paraburkholderia* sp.; 389C = *Pantoea cypripedii*; MIX = consortium of the four bacteria. The boxes represent the maximum and minimum values observed for each treatment. The black line represents the median and the red line, the mean. Different upper case letters above the columns indicate a significant difference between the means

Aloin content

The inoculation of the *A. vera* seedlings with the endophytic bacteria 35V *Enterobacter ludwigii* promoted a greater accumulation of aloin in the leaf gel (Figure No. 5a), with the mean content (3.40 ± 0.09 mg g-1) being significantly greater than that of the control plants (2.45 ± 0.11 mg g^{-1}), that is, a 38.77% increase. The highest mean alone content of the latex was also recorded in the plants inoculated with 35V *Enterobacter ludwigii* (230.21 ± 6.41 mg g^{-1}), increasing significantly, by 3.16%, in comparison with the control (223.15 ± 5.39 mg g^{-1}). However, the

aloin content of the latex was also significantly higher than the control in the plants inoculated with 135V *Enterobacter tabaci* (225.15 ± 0.93 mg g^{-1}) and the MIX (229.80 ± 5.00 mg g^{-1}) (Figure No. 5b). The alone content increased by 0.9% (in comparison with the control) in the seedlings inoculated with 135V *Enterobacter tabaci*, and by 2.98% in the MIX treatment.

DISCUSSION

The 149H *Paraburkholderia* sp. isolate, associated previously with the solubilization of Bayóvar phosphate and antibiosis to *Fusarium* sp., was efficient in promoting the growth of the *A. vera* plants, inducing leaf production and a greater accumulation of fresh biomass in the leaves and biomass in the roots. *Paraburkholderia* species have already been shown to promote growth in a number of other plants (Bolívar-Anillo *et al*., 2016; Huo *et al*., 2018; Zhou *et al*., 2018; Kuramae *et al*., 2020; García *et al*., 2020; Souza *et al*., 2020; Herpell *et al*., 2020). These bacteria are known to fix nitrogen (De Meyer *et al*., 2018; Tapia-García *et al*., 2020; Paulitsch *et al*., 2020), solubilize phosphate, and have antifungal activity (Bernabeu *et al*., 2018; Gao *et al*., 2018b; Hye-Jin & Min-Ho, 2019). These bacteria are capable of colonizing both the rhizosphere and the endosphere, have a diverse genome, and a wide range of physiological functions. In addition to promoting growth, these bacteria vitalize many plant species and maximize their tolerance of both abiotic and biotic stress (Mitter *et al*., 2013). This may account for the increase in biomass observed in the *A. vera* plants. In a study of micropropagated *A. vera* plants colonized by the symbiotic fungus *Piriformospora indica*, Sharma *et al*. (2014) observed a 16.5% increase in the gel content of the plants under *in vitro* conditions. In the present work, a 51.8% increase in leaf fresh content was obtained using 149H *Paraburkholderia* sp. as an inoculate.

In general, the latex of *A. vera* contains a larger amount of aloin than the gel, as noted by Sánchez-Machado *et al*. (2017), although the inoculation with a strain shown to produce IAA (135V *Enterobacter tabaci*) increased the aloin content in the latex of the *A. vera* plants. Spaepen & Vanderleyden (2011) concluded that the growth-promoting effect of the bacteria by phytostimulation through the production of IAA occurs through multiple mechanisms, such as N fixation, phosphate solubilization, and ACC deaminase activity, and results in the promotion of plant growth and increased yield. The IAA alters the root morphology and increases the absorption of water and nutrients from the soil (Castanheira *et al*., 2017), which favors plant growth (Liu *et al*., 2016). Auxin plays a number of roles related to changes in plant division, extension, and cell differentiation (Spaepen *et al*., 2007). This substance increases the development of the root and the xylem, as well as the biosynthesis of number of metabolites (Glick, 2012). In the plants inoculated with 135V *Enterobacter tabaci*, however, there was a trade-off between reduced root development and increased aloin content.

In the plants inoculated with 35V *Enterobacter ludwigii*, an isolate selected for its multifunctional potential (solubilization of Bayóvar phosphate, $Ca_3(PO_4)_2$ solubilization, antibiosis to *S. sclerotiorum* and IAA synthesis), a positive effect on root development was observed, associated with increased aloin content in the gel and latex. As for the plants inoculated with 135V *Enterobacter tabaci*, a trade-off was observed between the reduced accumulation of dry biomass and the increase in aloin content. The establishment of a symbiotic relationship may act, at least in the initial stages, as a stressful process for the plant, which would generate this trade-off. Cardarelli *et al*. (2013) demonstrated that saline stress, as well as AMF inoculation, increases the content of aloin and β-polysaccharides in both *Aloe arborescens* and *Aloe barbadensis*, while reducing many biometric parameters. On the other hand, Gupta *et al*. (2012) inoculated *A. vera* with rhizospheric bacteria isolated from the plant itself and obtained positive results in both the growth and alone content, with the best results being obtained with a consortium of the strains of *Pseudomonas synxantha*, *Burkholderia gladioli*, *Enterobacter hormaechei* and *Serratia marcescens*. In the present study, by contrast, the consortium treatment (MIX), which mixed the four bacteria (35V *Enterobacter ludwigii*, 135V *Enterobacter tabaci*, 149H *Paraburkholderia* sp. and 389C *Pantoea cypripedii*) obtained satisfactory results only for the aloin content in the latex. Further research will be required to determine the ideal combination of these strains, which may include the exclusion of 389C *Pantoea cypripedii*, which presented a reduced potential for the promotion of growth or the stimulation of aloin synthesis.

The results of the present study indicate that it is possible to augment the productivity of *A. vera*, with a 51.8% increase in the accumulated biomass following inoculation with 149H *Paraburkholderia* sp. and a 38.8% increase in the aloin content of the gel after inoculation with 35V *Enterobacter ludwigii*. It will nevertheless be necessary to evaluate the exact objective of the treatment, whether it should be aimed at increasing biomass or producing metabolites. Our findings indicate that the combination of 149H *Paraburkholderia* sp. and 35V *Enterobacter ludwigii* would both promote growth and increase the accumulation of aloin in the tissues. Biotechnological techniques focused on increasing *A. vera* productivity are scarce, and work is still focused on managing the supply of water and nutrients (e.g., Babatunde & Yongabi, 2008; Silva *et al*., 2010; Singh *et al*., 2021). This work demonstrates that the use of low-cost inputs such as microbial strains can be an important mechanism, contributing to improve the cultivation techniques associated with *A. vera*.

The understanding of the interactions between plants and microorganisms provides important insights for the selection of endophytes in the search for higher concentrations of the target metabolites and increased productivity, which will provide larger amounts of raw material for the manufacture of natural medicinal products. To satisfy the demand for medicinal plants from the manufacturing industry, the large-scale multiplication of the output, based on both rapid growth rates and higher productivity, is required, and this may be achieved, in part, by the application of multifunctional microorganisms.

CONCLUSIONS

We confirmed the hypothesis that endophytic bacteria isolated from *A. vera* promote growth and increase the aloin content in the leafy tissue when inoculated artificially in this plant. The root endophytic bacterium 149H *Paraburkholderia* sp., isolated from *A. vera*, maximized the accumulation of biomass by this plant, and could be applied as an inoculant to increase gel productivity, while the 35V *Enterobacter ludwigii* bacterium contributed to a significant increase in the aloin content of both the gel and latex of the *A. vera* plants, and can thus be recommended as an inoculate for the purpose of obtaining this metabolite. Further tests of the association between these two isolates should be conducted out in order to verify the potential for the promotion of growth linked to the synthesis of aloin.

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