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Effect of rhizobacteria isolated from *Suaeda* sp. in the growth of *Arabidopsis thaliana* and *Solanum lycopersicum* L. (Sahariana)
Efecto de rizobacterias aisladas de *Suaeda* sp. en el crecimiento de *Arabidopsis thaliana* y *Solanum lycopersicum* L. (Sahariana)

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SUMMARY

The rhizosphere of the great diversity of plants is a complex ecosystem that houses thousands of rhizobacteria that promote plant growth. In the current investigation, three bacteria were isolated from the root of *Suaeda* sp., which were evaluated to determine the effect of their inoculation on *Arabidopsis thaliana* at distances of 2 and 5 cm and in divided boxes. In the 2 cm test, we noticed that Endo10(7) and Endo10(5) stimulated the plants more than the control, while the proximity to Ecto10(6) caused them to wilt and die. However, at 5 cm, the bacterium that most promoted the development of *Arabidopsis* was Ecto10(6). In the divided box test, all three bacteria showed the ability to promote growth. In addition, a shade mesh assay was carried out with the inoculation of the bacteria in *Solanum lycopersicum* L. (Sahariana) and it was found that the promoting effect was also observed in the germination and growth of tomato plants. Tests were conducted to determine its ability to produce IAA, siderophores, and solubilize phosphates. Through molecular techniques it was confirmed: the identity of Ecto10(6), Endo10(7), and Endo10(5) as *Aneurinibacillus migulanus*, *Staphylococcus* sp. and *Bacillus cereus*, respectively. Our results provide the rationale for suggesting that these rhizobacteria may increase the growth and development of *Arabidopsis thaliana* and *Solanum lycopersicum*.

Index words: *IAA, phosphate solubilization, siderophores, PGPR, rhizosphere.*

RESUMEN

La rizósfera de la gran diversidad de plantas que existen, es un complejo ecosistema que alberga miles de rizobacterias promotoras de crecimiento vegetal. En la presente investigación se aislaron tres bacterias de la raíz de *Suaeda* sp., y fueron evaluadas para determinar el efecto de su inoculación en *Arabidopsis thaliana* a distancias de 2 y 5 cm y en cajas divididas. En el ensayo a 2 cm notamos que Endo10(7) y Endo10(5) estimularon más a las plantas que el control, mientras que la cercanía con Ecto10(6) les causó marchitez y muerte. Sin embargo, a 5 cm, la bacteria que fomentó más el desarrollo de Arabidopsis fue Ecto10(6). En el ensayo en cajas divididas las tres bacterias mostraron capacidad para promover crecimiento. Además, se realizó un ensayo en malla sombra con la inoculación de las bacterias en *Solanum lycopersicum L.* (Sahariana) y se constató que el efecto promotor también se observó en la germinación y crecimiento de las plantas de tomate. Se realizaron

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pruebas que establecieron su capacidad para producir AIA, sideróforos y solubilizar fosfatos. A través de técnicas moleculares se confirmó la identidad de Ecto10(6), Endo10(7) y Endo10(5) como *Aneurinibacillus migulanus*, *Staphylococcus sp*. y *Bacillus cereus*, respectivamente. Estos resultados nos dan el fundamento para sugerir que estas rizobacterias pueden incrementar el crecimiento y desarrollo de *Arabidopsis thaliana* y *Solanum lycopersicum*.

Palabras clave: *AIA, solubilización de fosfatos, sideróforos, RPCV, rizósfera.*

INTRODUCTION

Understanding the complexity of the soil microbiome remains a challenge. Plant growth promoting rhizobacteria (PGPR) are among the most studied because they positively change the environment and roots of plants, improving nutrient uptake and their development (Vejan, Abdullah, Khadiran, Ismail and Nasrulhaq, 2016). Some of the effects that these bacteria originate in the host plant include the increase in the germination rate, in the foliar area and stem and root growth (Panwar, Tewari, Gulati and Nayyar, 2016).

The microbiota that is associated with the plant has a very important role since it participates in increasing stress tolerance, using different mechanisms such as the accumulation of osmolytes, exopolysaccharide phytohormones, production of volatile compounds, modulation of the expression of related genes with stress, as well as inhibition and elimination of phytopathogens from the soil, in addition to contributing to the improvement of crop yield (Ali and Xie, 2020).

Salinity is the environmental stress that has the most prejudicial effects on plant growth and crop yields (Mayak, Tirosh and Glick, 2004). The halophytes can react in presence of salinity, either stimulating or inhibiting (Flowers and Colmer, 2008). These plants show adaptability to saline enviroments thanks to their associated microbiome and their genotype. For these reasons, this type of plant grows easily in dry regions of the world (Alexander, Mishra and Jha, 2019). Some studies demonstrated, that halophytes have succulence mechanisms that help them to maintain cell turgor pressure (Lokhande and Suprasanna 2012; Nikalje, Srivastava, Pandey and Suprasanna, 2018). Such is the case of the Suaeda plant, of which a series of works have increased knowledge of the interaction with its microbiome (Hidri *et al.,* 2022).

In the scientific community, *Arabidopsis thaliana* has been successfully used as a model plant, being ideal to work with it due to its small genome and its short life cycle (Goodman, Ecker and Dean, 1995). Different studies recognize their factibility for co-inoculation with beneficial bacteria in order to analyze the effects on plant development. Some recent results are reported by Ravelo-Ortega and López-Bucio (2022), García-Cárdenas, Ortiz, Ruiz, Valencia and López (2022) and Jiménez-Vázquez *et al*. (2020) who have described different mechanisms of interaction between microorganism-plant.

The use of PGPR is one of the ways towards food production with a more ecological and organic approach to environmental protection (Calvo, Nelson and Kloepper, 2014). Taking into account the great increase in the world population, the need for more sustainable agriculture that is capable of preserving our future becomes latent. Since today the world faces great challenges in relation to land degradation, the productivity of crops and the availability of fertile land are increasingly at stake. Just in Mexico 14% of the territory is dedicated to agriculture and in the coming years, friendly practices will play a crucial role in crop productivity and stability. The use and application of microbial inoculants over agricultural systems have been widely examined, such as the Pagnani *et al*. (2019) study demonstrates the positive influence on the growth, physiological and performance responses of wheat; which agrees with what was found by Cordero, Balaguer, Rincón and Pueyo (2018) confirming that PGPR can minimize the negative effects of abiotic stresses and arguing that this is the first step to guarantee sustainable agriculture worldwide.

Microorganism-plant interaction has long been of great interest since plants produce various compounds (sugars, organic acids, and vitamins) that microorganisms use as nutrients or signals. In turn, these microbial populations release phytohormones and volatile organic compounds that regulate plant growth and morphogenesis (Ortiz-Castro, Contreras, Macias and Lopez, 2009). It has previously been reported that different isolates belonging to the genus *Bacillus* have caused various effects on *A. thaliana* and favorably influenced its development (Palacio-Rodríguez *et. al*., 2017; López-Bucio, Acevedo, Ramirez, Molina and Herrera, 2006). Similarly, although less studied is the case for *Staphylococcus*, reported by Mora-Ruíz *et al*. (2018) where they showed that bacteria of the *Staphylococcus* genus promoted growth activity in *A. thaliana*.

Therefore, in the present investigation, the isolation of bacteria from the root of *Suaeda* sp was carried out, taking into consideration that the Comarca Lagunera is a region of great agricultural demand, however, currently it presents serious desertification problems. Therefore, farmers have seen the need to make excessive use of inorganic fertilizers, further affecting soil quality and crop yields. Consequently, it is important to evaluate and adopt best practices in relation to sustainable production. Therefore, the present study was developed where some biochemical characteristics that improve plant growth, the effect of its inoculation on the germination rate, and the growth promotion of *Solanum lycopersicum* L. (Saharan) were evaluated, selecting this crop by its great agricultural importance in the lagunera region, Mexico. Likewise, determine the identification of the strains by means of molecular techniques. Hoping that it leads to an increase in the development and exploitation of beneficial microbes to permit more sustainable agriculture. Based on the premise that the use of PGPR isolated from halophytes considerably increases plant development, the objective of this work was to evaluate the effect of inoculating three strains of PGPR on *Arabidopsis thaliana* and *Solanum lycopersicum* seedlings.

MATERIALS AND METHODS

The bacteria used in the present work were isolated in 2016 from the rhizosphere of *Suaeda* sp., a native plant of the Poza Salada in the Sobaco Valley, coordinates 102° 58′ 58″ W and 25° 45′ 32″ N, at a height of 1090 altitude meters (Czaja, Estrada and Flores, 2014), located in the municipality of San Pedro de las Colonias Coahuila, Mexico (Figure 1a,b).

For the isolation of the bacteria, a solution was prepared with one gram of plant root and 9 ml of 0.5X phosphate buffer saline (PBS), of which a serial dilution was made up to 10^{-3}; 100 µL was taken from each dilution and were inoculated separately in Petri dishes with Luria Bertani (LB) solid medium (Bertani, 1951), the incubation was for 24 h at 32 ± 2 degrees Celsius.

Three colonies with different morphology were selected, to which the identification keys were assigned: Ecto10(6), Endo10(7), and Endo10(5); they were then stored in suspension with 30% glycerol at -70 °C (Hunter and Belt, 1996).

In vitro Assays with *Arabidopsis thaliana*

Three *in vitro* experiments were carried out, with the objective of evaluating the growth promoting capacity of Ecto10(6), Endo10(7), and Endo10(5) in *Arabidopsis thaliana* (Col-0). Two of the tests were carried out to determine the effect of the inoculation distance of the bacteria in *A. thaliana* seedlings at 5 and 2 cm (distance and contact, respectively); and to analyze the efficacy of bacteria in promoting plant growth by means of volatile organic compounds (VOCs) a third test was developed in divided Petri dishes, inoculating the strain in a vertical line on one side, and the other side three *A. thaliana* seedlings were used.

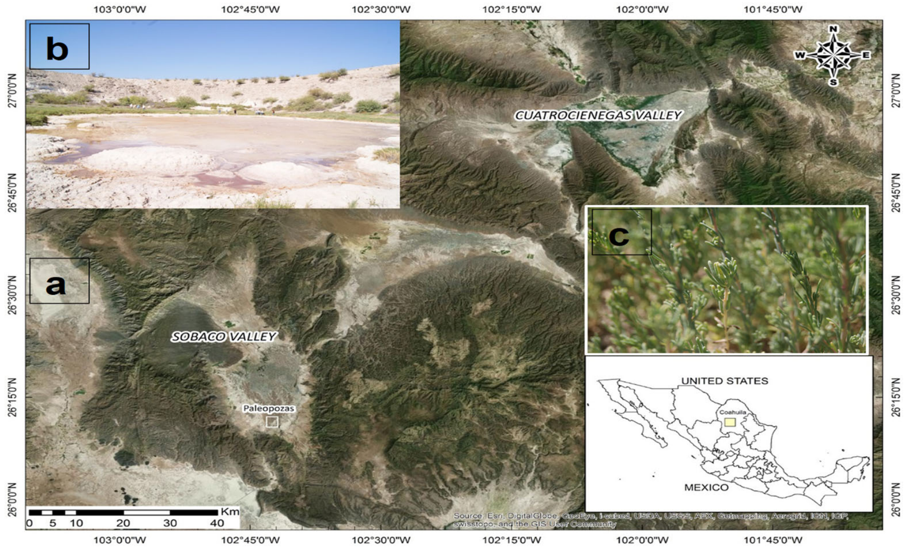

Figure 1. Prospecting the Chihuahuan Desert sampling area. (a) Geographic Location of the "Poza Salada" in the Sobaco Valley Coahuila, Mexico. (b) Sample collection site. (c) Halophyte *Suaeda* sp., grown near the "Poza Salada" body of water.

Seeds of *A. thaliana* (Col-0) were disinfected for seven minutes with 96% ethanol and 20% sodium hypochlorite for five minutes; To remove the chlorine residues, they were washed five times with sterile distilled water, then they were kept for two days at 4 degrees Celsius.

The seeds were germinated in Petri dishes with Murashige & Skoog (MS) 0.2X growth medium [1% (w/v) agar, 0.75% (w/v) sucrose, pH 7.0], kept for six days in a bioclimatic chamber, with conditions of 25 ± 1 °C, 16 h light/8 h darkness, with an inclination of 70° allowing the gravitropic growth of the root (López-Bucio *et al*., 2007).

The preparation of the inoculum was carried out as follows; The isolates were grown individually in LB liquid medium and placed in an incubator for 24 h at 29 ± 2 °C, with shaking of 200 rpm (Scientific Precision 815®), and the bacterial suspensions were adjusted to a concentration of 1×10^8 CFU mL^{-1} with PBS 0.5X (Espinosa-Palomeque *et al.,* 2017).

In the distance inoculation test, a series of six seedlings were placed per Petri dish with 0.2X MS medium, then the bacterial inoculum was applied horizontally, 5 cm below the tip of the *Arabidopsis* root. For the contact experiment, the bacterial suspension was first applied approximately 2 cm below the upper edge of the box, and six *A. thaliana* seedlings were placed on the inoculum (Gutiérrez-Luna *et al*., 2010).

To evaluate the VOCs, divided boxes were used, at one end the bacteria were inoculated and at the other, three *A. thaliana* seedlings were placed in parallel to the division of the box.

The experiments were carried out with three repetitions per treatment and were kept in the bioclimatic chamber (distributed by R.I.E.L. S.A. DE C.V.) with the conditions described above. The experimental unit consisted of each Petri dish. Twelve days after inoculation, the following parameters were evaluated: fresh weight, main root length, and number of secondary roots.

Trial with *Solanum lycopersicum* L. (Sahariana)

To determine the effect of the inoculation of the isolates in *S. lycopersicum*, two experiments were carried out in 2017: in the first, the stimulation or inhibition caused by the germination rate of the seeds was analyzed; the second was carried out to establish the influence of bacteria on tomato development in shade house conditions after 30 days of growing.

Solanum lycopersicum Var Sahariana seeds were used for the aforementioned experiments, which received a disinfection treatment with 96% ethanol for seven minutes and 20% sodium hypochlorite for five minutes, followed by five washes with sterile distilled water.

The suspensions of the bacteria were prepared and applied independently: Ecto10(6), Endo10(7), and Endo10(5) were grown in LB broth for 24 h and the concentrations were adjusted with PBS 0.5× to 1×10^8 CFU mL^{-1} (Espinosa-Palomeque *et al.,* 2017).

To evaluate the germination rate, the seeds were immersed in the suspensions for 2 h at 29 ± 2 °C, in an incubator with shaking of 2 rpm (Scientific Precision 815®); Subsequently, they were sown in a tray for germination (200 cavities of 3.1 cm × 3.1 cm wide, 7.0 cm high, 5 mm drainage) with a mixture of substrate peat moss: perlite: vermiculite in proportions 1: 1: 1 and they were kept at room temperature in the dark for seven days. The test was carried out with ten repetitions per treatment and the germination percentage was calculated.

In the second experiment with tomato, the treatment of the seeds was carried out in the same way as in the previous one. After germination, the seedlings were kept in a shade mesh for their development, with irrigation in the morning and at night, abundantly with running water. A second inoculation was carried out 20 days after sowing, before transplanting into the soil, the bacterial suspensions (prepared as described above) were applied (according to the treatment) in the area surrounding the roots of the plants, applying only morning watering. For its analysis, the following parameters were evaluated: plant height, root dry weight, number of lateral roots, foliar fresh weight, and foliar dry weight. The test was carried out with ten repetitions per treatment.

Mechanisms of Rhizobacteria

Each of the bacterial isolates was evaluated to establish some characteristics that could promote plant growth, for which several biochemical tests were carried out such as the production of indole acetic acid (IAA) with the addition of L-tryptophan, determined with the Salkowsky technique modified and described by Bric, Bostock and Silverstone (1991). The IAA concentration was quantified with a colorimetric method and compared with a standard curve (5, 25, 50, 75, and 100 µg mL^{-1}).

To test the production of siderophores, the Chrome azurol S (CAS) medium (Sigma-Aldrich, México DF) (Schwyn and Neilands, 1987) and the solubilization of phosphates with the selective culture medium NBRIP (National Botanical Research Institute Phosphate growth medium) (Nautiyal, 1999) were used. The positive confirmation for siderophore production was the formation of a yellow to orange halo surrounding the growth of the bacteria and the phosphate solubilization indicator was a clear halo. To measure the halos, a digital vernier (Carbon Fiber Composites Digital Caliper, Industrial & Scientific) was used and the results were evaluated by calculating the phosphate solubilization index (PSI) and siderophore production index (SPI) with the help of the PSI or SPI formula (as appropriate).

PSI or SPI = Diameter of the colony + solubilization halo / diameter of the colony (Edi-Premono, Moawad and Vleck, 1996).

The assay of all biochemical tests was made by three replications.

Statistical Analysis

The data obtained from the tests with *A. thaliana*, *S. lycopersicum* and rhizobacterial mechanisms were analyzed for normal distribution with the Shapiro-

Wilk test and then analyzed in a completely randomized experimental design and comparison of means with the Tukey test ($P \leq 0.05$). The program used for the analysis was GraphPad Prism version 6 (Motolusky, 1989).

16S rRNA Analysis

For their identification, the rhizobacteria were subjected to 16S rRNA sequence analysis. The extraction of chromosomal DNA was done from pure bacterial cultures, by means of the CTAB technique (Doyle and Doyle, 1990).

The purified PCR products were sent for sequencing at the Mc LAB laboratory in San Francisco, California, USA. Finally, the result was compared in the BLAST algorithm (Altschul *et al*., 1997) with the pre-established parameters in the NCBI database (NCBI, 1988).

RESULTS AND DISCUSSION

In the present investigation, the effect of our isolates of the genera *Bacillus* and *Staphylococcus* on the growth modulation of *A. thaliana* and *S. lycopersicum* was characterized. *Aneuirinibacillus migulanus*, *Staphylococcus* sp. and *Bacillus cereus* were able to stimulate the development of *A. thaliana*, exhibiting PGPR abilities. We were able to observe a positive effects test ($P \leq 0.05$) on *A. thaliana* in the in vitro assays in distance application. In the experiment carried out to evaluate the influence of the inoculation of Ecto10(6), Endo10(7), and Endo10(5) at a distance (5 cm), on the growth promotion of *A. thaliana* by diffusible compounds, it was observed that the leaf weight of the plants that had treatment with the bacteria showed increases of 109.3, 70.8 and 48.4% respectively, more than those of the control (Figure 2a-d) and when analyzing the development of the main root, those that were treated with Endo 10(7) had 28% more length than the plants of the other treatments and control (Figure 2e) ($P \leq 0.05$), in the case of the variables of the number of lateral roots and fresh weight, all the plants that had treatment with the bacteria were superior to those of the control however, Ecto10(6) benefited Arabidopsis more than other treatments (Figure 2f, g), ($P \leq 0.05$). These results suggest that there are probably growth regulating substances that act according to their proximity to the root system of the plants (Figure 2).

While in investigations carried out by García-Cárdenas *et. al.,* (2022) and Zhou *et al*. (2016) have shown the effect of co-cultivating isolates of the *Bacillus* and *Staphylococcus* genus in direct contact. Persello-Cartieux *et al*. (2001) studied the close relationship of AIA with the promotion and inhibition of root growth. And similar to what was reported by Jiménez-Vázquez *et al*. (2020), the application of the inoculum at a distance of 2 cm showed repressive effects in the case of *Aneurinibacillus migulanus*, which, being in contact with the plant, increased its production of compounds until it is covered, inhibiting its growth and subsequently causing wilting (Figure 3b), contrary to *Staphylococcus* sp. and *Bacillus cereus* that had the ability to stimulate the development of *A. thaliana* in direct contact (Figure 3a, c, d). In the main root length parameters Endo10(7) and Endo10(5) were 47 and 60% respectively, higher than the control, the number of lateral roots and fresh weight were 116.6 and 100% respectively, more quantity than those that do not have treatment, the plants inoculated with the bacteria had more promotion in these parameters compared to the control (Figure 3e, f, g) ($P \leq 0.05$). These results suggest that, although the direct application of *A. migulanus* is not suitable, it is possible that its promoting effect is regulated by bacterial metabolites. While probably in the case of *Staphylococcus* sp. and *B. cereus* could be the same or even some promotion by phytohormones.

Studies have been carried out in order to clarify the role of microbial VOCs during positive interactions between plants and microbes (Ortíz-Castro *et al*., 2009).

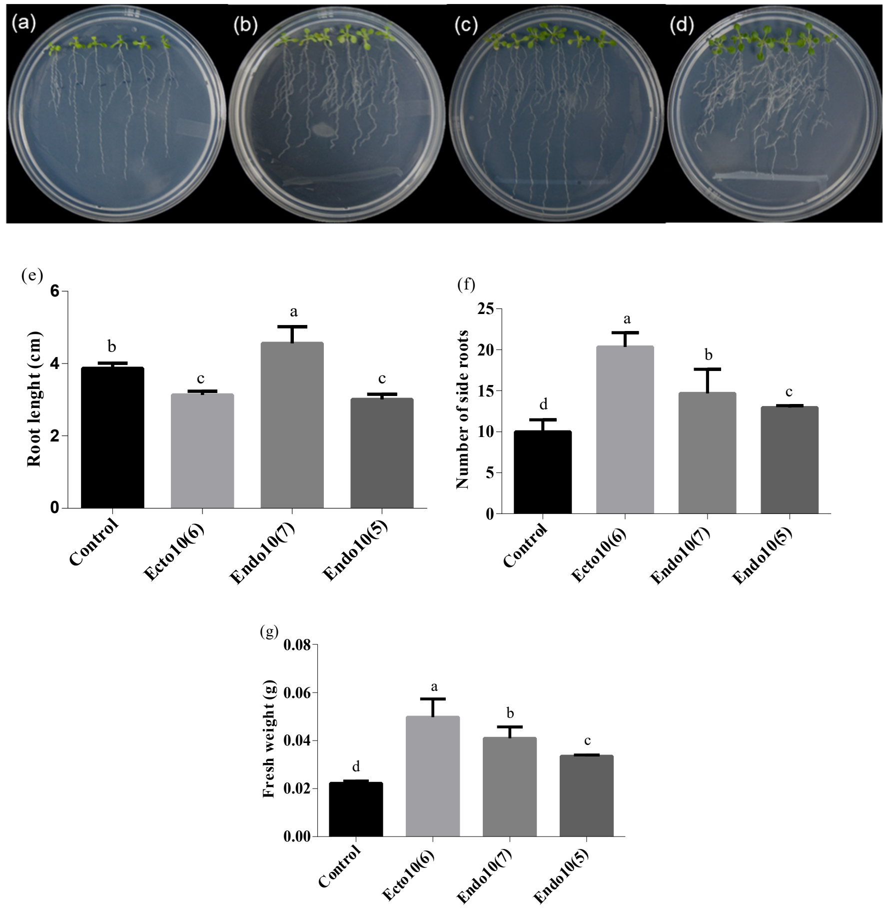

Figure 2. Effect of confrontation with, Ecto10(6), Endo10(7) y Endo10(5), in A*rabidopsis thaliana* (Col-0), by diffusible compounds (distance experiment), grown for 12 days in agar medium 0.2X MS, in (a) without bacteria (Control)., (b-d) Seedlings inoculated with *Aneurinibacillus migulanus*, *Bacillus cereus* and *Staphylococcus* sp., respectively., (e) Root length., (f) Number of side roots., (g) Fresh weight. † Values represent the mean ± standard deviation of 24 seedlings grown on four independent plates. † Different letters in the same column indicate significant differences, according to Tukey's test ($P \leq 0.05$).

Gutiérrez-Luna *et al.* (2010) reported isolates of *Bacillus cereus*, *Bacillus simplex,* and *Bacillus* sp., with the ability to increase plant and root development through VOCs. In our investigation it was shown that *Aneurinibacillus migulanus*, *Staphylococcus* sp. and *Bacillus cereus* favored the plant development of *A. thaliana* through the emission of VOCs (Figure 4a-d). Endo10(5) was better in the main root length parameter, increasing it by 63.5% (Figure 4e) ($P \leq 0.05$), however, the three bacteria influenced a greater number of lateral roots than the control (Figure 4f) ($P \leq 0.05$), increasing them 66.6% for Ecto10(6), 83.3% for Endo10(7) and 66.6% for Endo 10(5), finally, the inoculation with the latter increased the fresh weight of the plants 96.9, 90.0 and 106.9% respectively, compared to the control (Figure 4g) ($P \leq 0.05$).

Previously, plant promotion of plants such as *Arabidopsis* by means of VOCs produced by pathogenic bacterial genera, such as *Burkholderia cepacia,* and *Staphylococcus epidermidis*, has been reported (Spinelli *et al.*, 2011; Vespermann, Kai and Piechulla, 2007). In our study in the case of promoting plant growth due to the effect of volatile organic compounds (VOCs), both *Bacillus* and *Staphylococcus* were able to promote the development of *A. thaliana*. In most of the mechanisms that PGPR uses to interact with plants, the emission of VOCs plays a crucial role.

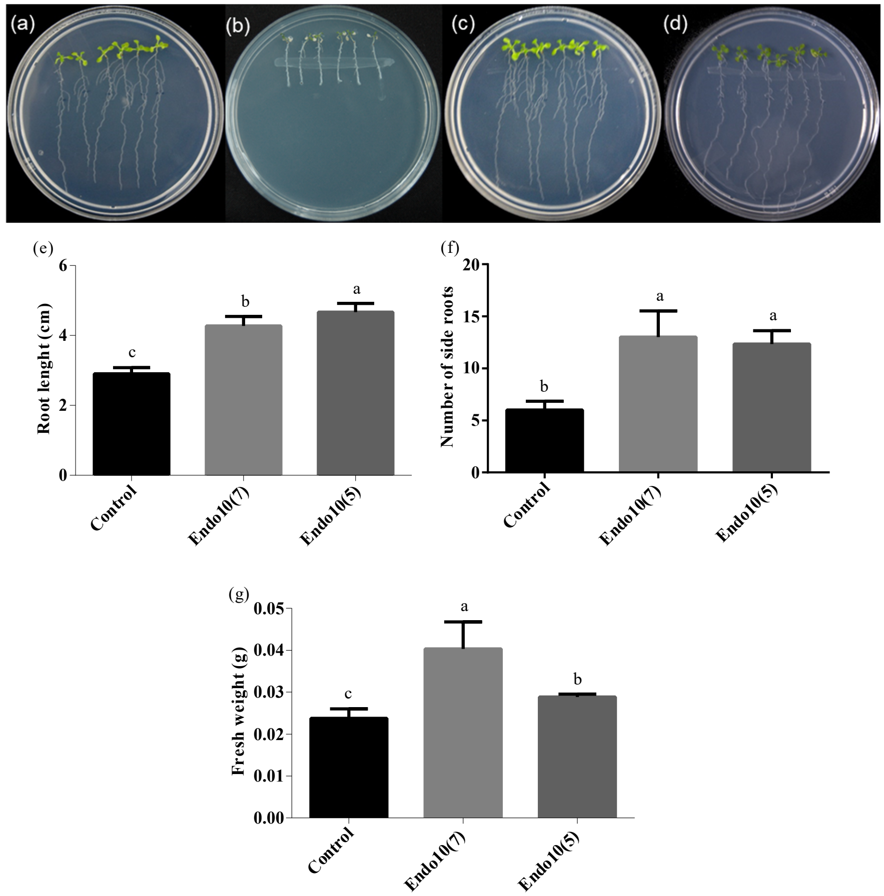

Figure 3. Effect of the confrontation with Ecto10(6), Endo10(7) and Endo10(5), in *A. thaliana* (Col-0), by direct contact (contact experiment), grown for 12 days in agar medium 0.2X MS, in (a) without bacteria (Control)., (b-d) Seedlings inoculated with *Aneurinibacillus migulanus*, *Bacillus cereus* and *Staphylococcus* sp., respectively., (e) Root length., (f) Number of side roots., (g) Fresh weight. † Values represent the mean ± standard deviation of 24 seedlings grown on four independent plates. † Different letters in the same column indicate significant differences, according to Tukey's test ($P \leq 0.05$).

Germination of *S. lycopersicum*

In the test that was carried out to determine the effect of the inoculation of the isolates on *S. lycopersicum* seeds, it was observed that those that had received inoculation treatment increased the percentage of germination between 10 to 45%, in relation to the seeds of the control ($P \leq 0.05$), which were the ones with the lowest proportion of germination 66%; followed by Endo10(7) with 73%; Endo10(5) with 86%, and the bacterium that provided the greatest stimulus to the seeds was Ecto10(6) with 96 percent.

Shade Mesh Trial With *S. lycopersicum*

Although no reference was found in tomato, the genus *Staphylococcus* has revealed the ability to promote growth through the regulation of ion balance and modulation of the corn response to salinity stress (Shahid *et al*., 2019); its application has also been related to increased yield in strawberry production (Ipek *et al.,* 2014). Mukhtar *et al.* (2020) observed that the application of *B. cereus* in *S. lycopersicum*

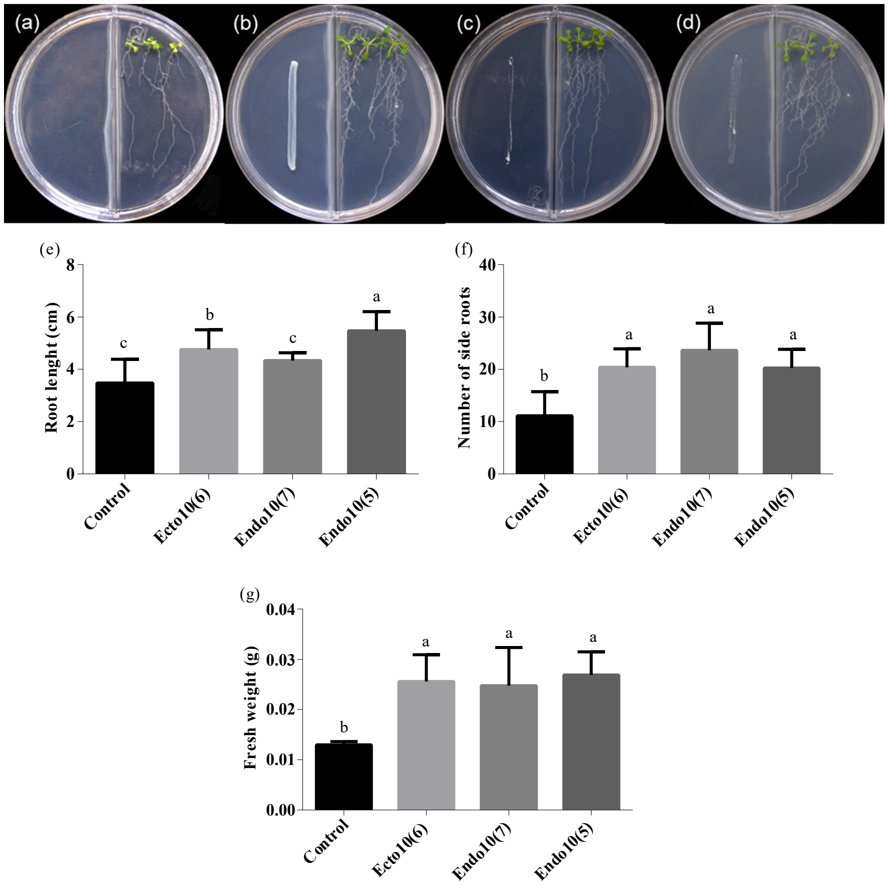

Figure 4. Effect of confrontation with Ecto10(6), Endo 10(7) and Endo10(5), in *Arabidopsis thaliana* (Col-0), by volatile organic compounds (VOC's), grown for 12 days in agar medium 0.2X MS, in (a) without bacteria (Control)., (b-d) Seedlings inoculated with Aneurinibacillus migulanus, *Bacillus cereus* and *Staphylococcus* sp., respectively., e) Root length., f) Number of side roots., (g) Fresh weight. † The values represent the mean ± standard deviation of 24 seedlings grown in four independent plates. † Different letters in the same column indicate significant differences, according to Tukey's test (*P* ≤ 0.05).

seedlings increased the length of its root, fresh and dry biomass and that they agree with those obtained in this study, in which the bacterium also shows promoting activity in these same parameters and our results also show that its inoculation has positively influenced the fresh and dry weight of tomato plants. In this trial (Figure 5a-d) it was observed that the efficacy of the inoculation of the isolates in *S. lycopersicum* was similar to that obtained in *A. thaliana*, relevant changes were produced in the morphology of the tomato seedlings, which could be due to the stimulation exerted by rhizobacteria, particularly in the variables: plant height, number of lateral roots, total fresh weight and total dry weight, which had higher development when compared to control plants. The height of the plants with the treatment of the isolates was notably higher, in 18.2% for Ecto10(6), 25.1% for Endo10(7), and 31.9% for Endo10(5) (Figure 5e) (*P* ≤ 0.05), in the case of the number of roots lateral Ecto10(6) increased 14.7% and Endo10(5) 17.6%, while Endo10(7) had similar results to the control (Figure 5f) (*P* ≤ 0.05); the variable dry weight of *S. lycopersicum* was increased 91.3% for Ecto10(6), 133% for Endo10(7), and 93.5% for Endo10(5) (Figure 5g, h). In the case of fresh weight, it was increased by 96.9% for Ecto10(6), 123.2% for Endo10(7), and 97.4% for Endo10(5) (Figure 5 i-j).

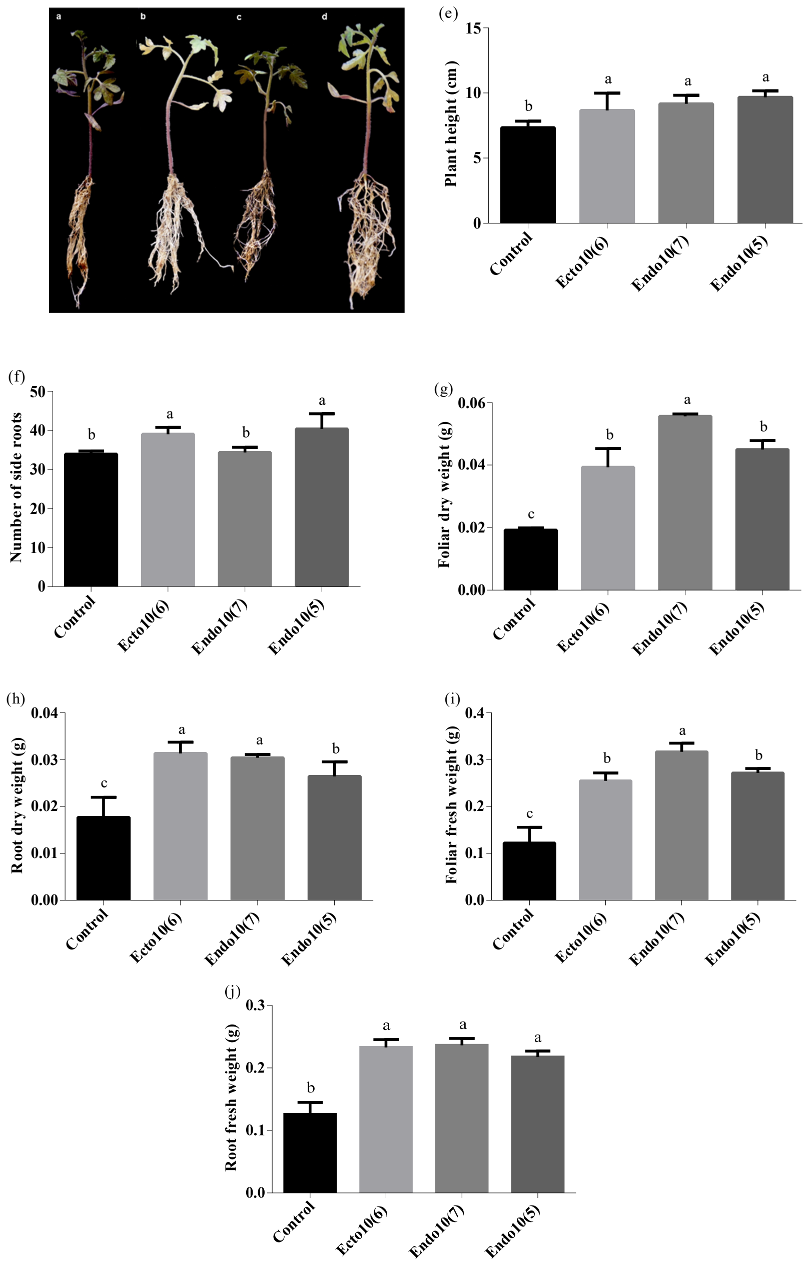

Figure 5. Effect of inoculation of Ecto10(6), Endo10(7) and Endo10(5), in seeds of S. *lycopersicm* (2 h), co-inoculated at 20 days of flooding and collected at 30 days. (a) Control without inoculation., (b-d). *A. thaliana* co-grown with *Aneurinibacillus migulanus*, *Bacillus cereus* and *Staphylococcus* sp., respectively., (e) Plant height., (f) Number of side roots., (g) Foliar dry weight., (h) Root dry weight., (i) Foliar fresh weight., (j) Root fresh weight. † The values represent the mean ± standard deviation of 40 seedlings. † Different letters in the same column indicate significant differences, according to Tukey's test ($P \leq 0.05$).

Mechanisms of Rhizobacteria

Among the most relevant characteristics of the isolates is the production of IAA from L-tryptophan. What was reported by Hassan and Bano (2015), where they analyzed an isolate of *B. cereus* with the addition of L-tryptophan, finding effectiveness with and without the addition of this, attributing it to the production of IAA and also agree with observed by Prakamhang *et al*., 2015, where its *Bacillus* sp. isolate was the largest producer of AIA. This is consistent with our *Bacillus cereus* isolate Endo10(5), which was able to obtain the highest amount of IAA with 15.12 µg ml^{-1}, followed by Endo10(7) with 7.21 µg ml^{-1}, the one with the lowest concentration was Ecto10(6) with 2.15 µg ml^{-1} ($P \leq 0.05$). Vega, Rodriguez, Llamas, Bejar and Sampedro (2019) observed an increase in shoot length and root length of tomato seeds with the inoculation of *Staphylococcus* EN21 compared to the non-inoculated control and in our investigation, the co-inoculation of Endo10(7) in *A. thaliana* showed an increase in root length, which coincides with what was previously found. This indicates that the strains have the ability to produce the indole acetic acid phytohormone using the L-tryptophan metabolic pathway, as a property of PGPR, as observed in the biochemical test for IAA production.

The production of siderophores is another of the mechanisms evaluated and the presence of these compounds in the CAS medium was exposed by the formation of an orange halo around bacterial growth, which was measured to obtain the production index of each bacterium; Endo10(7) had the highest index with 6.5 mm, Ecto10(6) had 2.36 mm and Endo10(5) with the lowest value with 2.25 mm ($P \leq 0.05$). The identification of Endo10(7) gave a similarity of 97.89% with *Staphylococcus* sp., and it was the one that showed a higher index in the production of siderophores, however, it has been little reported with this characteristic, an exception was in a group of bacteria isolated from barley and tomato, studied by Scagliola *et al*. (2016).

In the determination of phosphate solubilization *Bacillus brevis* has been previously reported among the potentially solubilizing species (Corrales-Ramírez, Sánchez, Arévalo and Moreno, 2014). The solubilization of phosphates determined through the formation of a clear halo surrounding the bacteria showed us that Ecto10(6), Endo10(5), and Endo10(7) had this ability, the values obtained were 1.67, 2.39, and 7.21 mm ($P \leq 0.05$). Being our isolated strain Endo10(7) was identified as *Aneurinibacillus migulanus*, which showed the most solubilization capacity.

16S rRNA Analysis

Using the NCBI database (NCBI, 1988), the sequences obtained were analyzed via BLAST, and by pairing it was determined that Ecto10(6) is similar to *Aneurinibacillus migulanus*, Endo10(5) is similar to *Bacillus cereus* and Endo10(7) is similar to *Staphylococcus* sp. (Table 1). Molecular identification will allow knowing the bacterial genera present in the collection sites and with the potential to be used as PGPR.

Table 1. Molecular identification using the 16S rDNA gene portion and BLAST sequence alignment from the NCBI database (NCBI, 1988).

Molecular identification of rhizobacteria				
ID	Nearby species	Identity	Access number NCBI	Bp
		%		
Ecto10(6) LB cs	*Aneurinibacillus migulanus*	98.41	KX083693.1	1385
Endo10(7) LB cs	*Staphylococcus sp.*	99.78	MT225634.1	1395
Endo10(5) LB cs	*Bacillus cereus*	98.69	MH985223.1	1377

CONCLUSIONS

The results of this study show that *Bacillus cereus*, *Aneurinibacillus migulanus,* and *Staphylococcus* sp. have characteristics that help promote plant growth by increasing root development, number of lateral roots and total weight, by activating biochemical mechanisms, such as phosphate solubilization, indole acetic acid production and siderophores, therefore, we can suggest their use as potential biological fertilizers capable of counteracting endogenous and exogenous limitations of plant growth. Although more tests should be done before being recommended as a fertilizer. These results also open the opportunity for future studies measuring the feasibility and effect of combining the characterized bacteria in order to demonstrate a broader coverage of response to plant stress.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Writing-original draft preparation and investigation: J.L.C.A. Methodology and resources: J.S.M. Writing-review and editing: M.A.G.R. and M.F.H.

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