



Short note [Nota corta]

**PLANT GROWTH PROMOTING RHIZOBACTERIA ISOLATED FROM
Solanum tuberosum L. IN VERACRUZ-MÉXICO RIZOBACTERIAS†**

**[RIZOBACTERIAS PROMOTORAS DE CRECIMIENTO AISLADAS DE
Solanum tuberosum L. EN VERACRUZ-MÉXICO]**

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SUMMARY

Background: The central area of the state of Veracruz is widely known as one of the regions with the largest hectares dedicated to the production of potato (*Solanum tuberosum* L.), this is susceptible to different diseases, an strategy of managements is with applications of growth-promoting rhizobacteria that have yielded favorable results in other crops. **Objective:** To evaluated seven bacterial strains isolated from the rhizosphere of *Solanum tuberosum* in terms of plant growth promotion (PGPR). **Methodology:** The isolates belonged to the species: *Providencia alcalifaciens*, *Pseudomonas syringae* pv *syringae*, *Serratia marcescens*, *Serratia liquefaciens* (I), *Serratia liquefaciens* (II), *Pseudomonas putida* and *Serratia ficaria*. Biochemical tests were performed such as production of siderophores, solubilization of phosphate, production of indole-3-acetic acid and nitrogen fixation. **Results:** All the bacterial strains functioned as plant growth promoters, compared to the negative control, particularly the *P. syringae* pv *syringae* and *S. liquefaciens* (I) strains. **Implications:** The use of these strains represent an alternative to improve the phytosanitary conditions of this crop for the area. **Conclusion:** Based on the above, we consider that the seven bacterial strains evaluated in this study constitute a promising and environmentally friendly strategy in development of potato crop of Perote Veracruz.

Keywords: biochemical tests; phosphate solubilization; PGPR; production indolic compounds (IAA); rizosphere; siderophores.

RESUMEN

Antecedentes: La zona centro del estado de Veracruz es ampliamente conocida como una de las regiones con mayor cantidad de hectáreas dedicadas a la producción de papa (*Solanum tuberosum* L.), esta es susceptible a diferentes enfermedades, una estrategia de manejo es la aplicación de rizobacterias promotoras de crecimiento que han tenido resultados favorables en otros cultivos. **Objetivo:** Evaluar siete cepas bacterianas aisladas de la rizosfera de *S. tuberosum* en términos de promotoras de crecimiento de las plantas (PGPR). **Metodología:** Los aislamientos fueron las especies: *Providencia alcalifaciens*, *Pseudomonas syringae* pv *syringae*, *Serratia marcescens*, *Serratia liquefaciens* (I), *Serratia liquefaciens* (II), *Pseudomonas putida* y *Serratia ficaria*. Se realizaron pruebas bioquímicas como: producción de sideróforos, solubilización de fosfato, producción de ácido indol-3-acético y fijación de nitrógeno. **Resultados:** Todas las cepas bacterianas funcionaron como promotoras del crecimiento de las plantas, en comparación con el control negativo, particularmente las cepas *P. syringae* pv *syringae* y *S. liquefaciens* (I). **Implicaciones:** La

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utilización de estas cepas representan una alternativa para mejorar las condiciones fitosanitarias de este cultivo para la zona. **Conclusión:** Con base en lo anterior, consideramos que las siete cepas bacterianas evaluadas en este estudio constituyen una estrategia promisoriosa y amigable con el ambiente para el desarrollo del cultivo de papa de Perote Veracruz.

Palabras claves: Pruebas bioquímicas; solubilización de fosfato; PGPR; producción de compuestos indólicos (IAA); rizosfera; sideroforos.

INTRODUCTION

Plants establish symbiotic relationships with soil microorganisms during their growth and development in an eco-friendly way (Gouda *et al.*, 2018). The rhizobacteria, plant growth promoting, protect plants from diseases and abiotic stress by diverse mechanisms, including the increase in the bioavailability of nutrients such as, nitrogen fixation and phosphate solubilization, the alleviation of abiotic stress by modulating the 1- aminocyclopropane-1-carboxylate deaminase expression and the production of phytohormones, Siderophores induced Systemic Resistance, (Souza *et al.*, 2015).

Positive effects of rhizosphere microbiota have been widely studied for their role in plant health as antagonist, and as growth promoters for their nitrogen and mineral fixation ability in an environmentally sustainable way (Hernández-Rodríguez *et al.*, 2006; Munees and Khan, 2011; Kloepper *et al.*, 1980; Tkacz and Poole, 2015). Therefore, there is a growing interest in the search of novel environmentally friendly alternatives, to mitigate damage caused by pathogens and attain better crop yields (Reyes *et al.*, 2008) like the use of rhizobacteria as plant growth promoters. Inoculating the crops with PGPR could substantially reduce the use of synthetic fertilizers and the negative impacts to the soil, contributing to the producer's economy and the population's nutrition (Moreno-Reséndez *et al.*, 2018).

Potato production is challenged by environmental factors such as climatic conditions, water availability, soil characteristics, pests and diseases. To maintain high productivity levels and increase of agrochemical inputs is required, especially of nitrogen fertilizers and pesticides (Olle *et al.*, 2015; Sifuentes-Ibarra *et al.*, 2013).

The excessive application of fertilizers applied by potato farmers in the Perote region to the main varieties that are cultivated such as Vivaldi, Fiannas, Tollocan, Alfas Gigant, Adoras, Felcinas, cause socioeconomic and environmental problems, for example; high financed costs in chemical fertilizers for increase yields, as consequence the soils is degraded more quickly, and therefore become be less fertile (Fernández, 2011).

In this research, seven bacterial were isolated from potato rhizosphere and tested as PGPR with the

objective of used ecologically friendly alternatives for production of this crop.

MATERIAL AND METHODS

Bacterial isolation and molecular identification

Bacteria strains were previously isolated and identified by Salinas-Castro *et al.* (2016), finally was carried out sequencing of 16S rRNA They were identified as *Providencia alcalifaciens* Ewing, *Pseudomonas syringae* pv. *syringae* Van Hall, *Serratia marcescens* Bizio, *Serratia liquefaciens* SL (I), *Serratia liquefaciens* SL (II), *Pseudomonas putida* Trevisan and *Serratia ficaria* Bizio.

Biochemical tests

For quantify the production indolic compounds (IAA), each strain was previously cultured on nutrient glucose agar (NGA). Then, colonies were suspended on saline solution (0.85 g NaCl/100 mL H₂O) to between 10⁵-10⁶ CFU/mL. The inoculum was diluted on trypticase soy broth (TSB) and incubated at 30 °C for 96 h, under constant agitation (120 rpm). Aliquots of 2 mL were then centrifuged for 10 min at 5000 rpm and the indole compound production was measured as IAA equivalents by taking 1 mL of supernatant and mixed with 2 mL of Salkowski reagent, incubated for 30 min at room temperature in darkness and recording absorbance at 530 nm by a UV/VIS spectrometer (Perkin-Elmer Lambda 40), using a standard curve for calibration for IAA, one positive control and negative control (Clavijo *et al.*, 2012).

Bacteria samples were evaluated for their capacity of siderophores production in Petri azulol S agar (CAS) as described for Loudon *et al.*, (2011). For this, bacteria grew in NGA at 30 °C for 48 h and then, colonies were resuspended on saline solution (0.85 g NaCl/100 mL). Then 10 µL micro drops of the suspension were cultured on Petri dishes that contain chrome azulol S agar for 7 days at 30 °C. Bacteria that produce siderophores formed a yellow halo around the colonies in the blue-green media.

Strains isolated that solubilize phosphates were identified as described by Chen *et al.* (2006) and Nauyital (1999). Bacteria were grown in medium using tricalcium phosphate as sole phosphorus source.

Strains were incubated for 12 days at 28 °C strain that formed a visible clearing halo around colonies. Were considered phosphate solubilizers in relation to control strain *Escherichia coli* Escherich, a known phosphatase solubilizer isolate.

The nitrogen fixing ability was tested using the method proposed by Döbereiner *et al.* (1999). Strains were inoculated in 10 mL vials with semiliquid nitrogen fixation medium (NFB), leaving the negative control without inoculum. Vials were incubated during two weeks at 30 °C. The positive reaction was manifested as a halo like bacterial growth.

Inoculation of potato tubers with bacterial strains

Tubers of *S. tuberosum* L. cv. Fianna were surface disinfested with 4% sodium hypochlorite for 30 s and rinsed with autoclaved distilled water and inoculated with *P. alcalifaciens* Ewing, *P. syringae* pv. *syringae* Van Hall, *S. marcescens* Bizio, *S. liquefaciens* SL (I), *S. liquefaciens* SL (II), *P. putida* Trevisan and *S. ficaria* Bizio. For the inoculation, potato tubers were sprayed at seven days interval using a bacterial strain at a concentration of 1.2×10^8 CFU/mL and kept under refrigeration at 8 °C for a total of twenty-one days with the purpose to allow bacteria colonize the tubers. Control plants were inoculated with autoclaved distilled water.

Plastic boxes of 40 x 60 x 30 cm were filled with 15 kg of sterile soil and inoculated tubers were planted. Five replications were maintained for each isolated strain. Plants were grown in a greenhouse established at Las Vigas de Ramirez, Veracruz, Mexico (19° 38' N, 97° 38' W; at an altitude of 2,420 m.a.s.l.) under a natural day/night cycle in April 2016 for approximately 3 months at 25 °C. Thirty days after the sowing, each experimental unit was inoculated three times with 500 mL of bacterial solutions at a concentration of 1.2×10^8 CFU/mL every 15 days by spraying. At harvest in July, the following variables were evaluated: yield of tubers and fresh and dry weight of foliage. Dry weight was measured after oven-drying foliage for 15 days; plant height was measured with a millimeter ruler.

Statistical analysis

The experimental design was a completely randomized block with seven treatments, five repetitions and an untreated control. Data were analyzed by one-way ANOVA followed by a Tukey tests, with a significance level of 0.05. Statistical analysis was performed with R v3.2.1 (R Core Team, 2015).

RESULTS AND DISCUSSION

Among the strains tested only *S. ficaria* produced siderophores (Table 1) which exerted the greater effect on promotion in the greenhouse assay, suggesting that its activity might be related. Siderophores are low molecular weight bio-molecules secreted by microorganisms in response to iron starvation because of acquisition of iron from insoluble forms by mineralization and sequestration. The ability to produce siderophores not only improve rhizosphere colonization of producer strain but also plays an important role in iron nutrition and antagonism against phytopathogens being a desirable trait for potential PGPR (Aruna-Sharmili *et al.*, 2015).

Table 1. Siderophores production and nitrogen fixation of *S. liquefaciens* I, *S. marcescens*, *P. syringae*, *P. alcalifaciens*, *S. ficaria*, *S. liquefaciens* II and *P. putida* isolated from potato rhizosphere.

Bacterial strain	Siderophore production	Nitrogen fixation
<i>P. putida</i>	(-)	(+)
<i>P. syringae</i> pv <i>syringae</i>	(-)	(+)
<i>S. ficaria</i>	(+)	(+)
<i>P. alcalifaciens</i>	(-)	(+)
<i>S. liquefaciens</i> I	(-)	(+)
<i>S. liquefaciens</i> II	(-)	(+)
<i>S. marcescens</i>	(-)	(+)
Negative control (Culture medium NFB)		(-)

P. syringae pv *syringae* produce the highest amount of IAA (24 µg/mL) and *S. ficaria* the lowest (9 µg/mL) (Table 2). This production of IAA resulted in the highest average yield of tubers rhizobacteria has been reported to fluctuate from 2 to 50 µg/mL, depending on the strain and growth conditions (Clavijo *et al.*, 2012). There is for the same strain IAA produce some controversy about the impact of hexogen auxins on plant development, since there have been documented both positive and negative effects. The consequences for the plant depend usually on two factors: (1) the amount of IAA available for the plant, and (2) the sensitivity of the vegetable tissues to changes in IAA concentrations (Angulo *et al.*, 2014).

The seven bacterial strains showed ability for phosphate solubilization. Species from genera *Pseudomonas* and *Serratia* showed the best ability, manifested by the presence of a 5 to 20 mm halo in the culture medium. The wider halo corresponded to the strains *P. syringae* pv *syringae*, *P. putida* and *S. liquefaciens* I. When the phosphate solubilization is evaluated *in vitro*, it is foreseen its potential effect on the absorption of this nutrient by the plant, suggesting

the possibility of the agricultural use of such bacteria (Reyes *et al.*, 2006; Mrkovački and Milic, 2001). There are several examples, that stand for the benefits of the application of phosphate solubilizing bacteria (PSB) on crops of economic importance, such as the study reported by Sánchez-López *et al.*, (2012), in which tomato plants (*Solanum lycopersicum* L.), were inoculated with *Pseudomonas putida* PSO14 and *Enterobacter sp.* TVL-2 in greenhouse conditions, reaching yield increments between 17 % and 49% (Almaghrabi *et al.*, 2013).

Table 2. Indole acetic production ($\mu\text{g/mL}$) of *S. liquefaciens* I, *S. marcescens*, *P. syringae*, *P. alcalifaciens*, *S. ficaria*, *S. liquefaciens* II and *P. putida* isolated from potato rhizosphere. (n=9).

Treatments	Indole acetic production	Mean IAA	Standard error
Control+	53.52	5.07 a	0.0000633
<i>P. syringae</i> pv. <i>syringae</i>	24	2.34 b	0.00197
<i>S. liquefaciens</i> II	23	1.92 c	0.00102
<i>P. putida</i>	21	1.82 d	0.000794
<i>S. liquefaciens</i> I	14	1.35 e	0.0158
<i>S. marcescens</i>	12	0.91f	0.000177
<i>P. alcalifaciens</i>	10	0.80 g	0.000209
<i>S. ficaria</i>	9	0.79 g	0.00144
Control (-)	0.3	0.45 h	0.0165

Different letters in columns represents significant differences Tukey's test ($P \leq 0.05$).

In a study done by Gervasio *et al.*, (2019) with plant growth promoting rhizobacteria, found beneficial effects on crops, the objective of the work was to select a strain of *Rhizobium* genus able to stimulate growth in potatoes. To this end, work was carried out under semicontrolled conditions and the use of two strains

Table 4. Effect of the application of *S. liquefaciens* I, *S. marcescens*, *P. syringae*, *P. alcalifaciens*, *S. ficaria*, *S. liquefaciens* II and *P. putida* isolated from potato rhizosphere on potato cultivation's fresh foliage weight and dry foliage weight (g) in greenhouse.

Treatments	Mean fresh foliage weight	Standard error	Mean dry foliage weight	Standard error
<i>P. putida</i>	87.60 a	20.47	1.25 a	0.15
<i>S. liquefaciens</i> II	66.20 ab	7.51	1.09 a	0.10
<i>P. alcalifaciens</i>	65.00 ab	17.36	1.00 ab	0.19
<i>S. ficaria</i>	52.60 ab	1.83	0.81 ab	0.08
<i>S. marcescens</i>	49.20 ab	4.50	0.90 ab	0.06
<i>P. syringae</i> pv <i>syringae</i>	46.00 ab	5.63	0.90 ab	0.07
<i>S. liquefaciens</i> I	43.60 ab	3.31	0.90 ab	0.07
Untreated control	30.60 b	3.90	0.66 b	0.09

Different letters in columns represents significant differences (Tukey, $p= 0.05$). Negative control: treated with distilled water.

(C1: *Rhizobium etli* CE-3 and C2: *Bradyrhizobium elkani* ICA 8001) as well as two doses of each (D1: 3 μL and D2: 6 μL) applied in the niche in which the tuber was placed at the time of planting. In all the evaluated variables, the height of the plants, the number of stems, the number of stolons emitted by tubers, the average number of leaves per stem, the strain *Bradyrhizobium elkani* ICA 8001, was the one that favored the best result with significant differences respect to the control.

Table 3. Efficacy of potato rhizosphere bacteria *S. liquefaciens* I, *S. marcescens*, *P. syringae*, *P. alcalifaciens*, *S. ficaria*, *S. liquefaciens* II and *P. putida* to enhance potato yield (kg/pot).

Treatments	Actual yield (kg/pot)	Yield increase (%)
<i>P. syringae</i> pv <i>syringae</i>	1.57 ± 0.12^a	61.86
<i>S. liquefaciens</i> I	1.49 ± 0.03^a	53.61
<i>S. marcescens</i>	1.36 ± 0.08^{ab}	40.21
<i>S. liquefaciens</i> II	1.30 ± 0.06^{ab}	34.02
<i>P. putida</i>	1.25 ± 0.07^{ab}	28.87
<i>P. alcalifaciens</i>	1.24 ± 0.12^{ab}	27.84
<i>S. ficaria</i>	1.23 ± 0.09^{ab}	26.80
Negative Control	0.97 ± 0.02^b	---

Different letters in columns represents significant differences (Tukey, $p= 0.05$). Negative control: treated with distilled water.

The efficacy of isolated bacteria as plant growth promoters in potato is shown in Table 3 where *P. syringae* pv. *Syringae* and *S. liquefaciens* I obtained the best result in production of tubers in the experiments established. The other strains did not show any significant differences between the treatments; however, there was a higher yield (between 21% and 29%) than the control. The dry and fresh weight of the foliage increased in a significant manner with the treatment given with *P. putida* (Table 4).

Nitrogen fixing, all strains presented a positive response (Table 1). Similarly, there is a growing wealth of knowledge suggesting the nitrogen fixation capacity of *Pseudomonas spp.* In different crops and environments. Those reports seem coincident with the results of our study, since *P. putida* was found to increase the fresh and dry weight of the greenhouse assay (Table 4).

However, nitrogen fixation is an energetically expensive process for bacteria, although it represents a major competitive advantage for them, so the whole process depends mainly in the availability of such element in the soil (Orozco-Jaramillo and Martínez-Nieto, 2009). *P. syringae pv. syringae* and *S. liquefaciens* (I) showed the best potato average yields. Almaghrabi *et al.* (2013) found that *S. marcescens* showed greater efficiency as PGPR in greenhouse tomato plants, 45 days after inoculation with nematodes of the genus *Meloidogyne*, analyzing variables, dry weight, plant height, plant yield and fewer $J_2/10$ g soil, compared to treatments *Pseudomas putida*, *P. fluorecens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. ceurus*. Simultaneously, *P. fluorescens* and *P. putida* have been reported to stimulate plants growth and increment the yield of cultivation through their antagonism against potential phytopathogens (Kloepper *et al.*, 1980). On the other hand, *Pseudomonas syringae* is capable of synthesize the indole-acetic acid departing from the tryptophan through indole-3-acetamide (Baca and Elmerich, 2007; El Sorra *et al.*, 2007).

CONCLUSION

Finally, we concluded that the seven bacterial strains possess desirable traits to be used as PGPR. However the best results in greenhouse assay were achieved by *P. syringae pv. syringae* and *S. liquefaciens* (I). Based on the above, we consider said bacterial strains to constitute a promising and environmentally friendly strategy in the potato crop of Perote Veracruz.

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Conflict of interest. We have no conflict interest to declare.

Compliance with ethical standards. It does not apply.

Data availability. Data are available with Alejandro Salinas-Castro, asalinas@uv.mx upon request.

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