

Isolation and selection of rhizospheric bacteria with biofertilizing potential for corn cultivation

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ABSTRACT

Objective: To isolate and determine in a greenhouse environment the biofertilizing potential of rhizospheric bacteria associated to corn (*Zea mays* L.) at Campeche, Mexico.

Design/methodology/approach: Rhizospheric soils were collected from two corn production zones with different management conditions. Bacterial strains were isolated from these samples and their biofertilizing potential determined by *in vitro* and *in vivo* tests. The obtained data from both tests were assessed using an analysis of variance (ANOVA) and a means comparison test (LSD, $p \leq 0.01$).

Results: In total, 16 rhizospheric bacteria were isolated, a higher number in non-mechanized soils ($n=10$) compared to mechanized ones ($n=6$). In the *in vitro* tests, the most representative activity corresponds to nitrogen fixation (81%) attributed to a higher bacteria percentage, while the activity with lower bacteria numbers corresponds to IAA production (25%). At the *in vivo* tests in corn plants, the YM1 strain presented the highest fresh and dry root biomass (20 and 2 g plant⁻¹, respectively). The YM4 strain promoted greater plant height (63.33 cm), and YM5 registered the highest values in stem diameter (7.13 mm), root length (36.78 cm) and fresh shoot weight (12.03 g plant⁻¹).

Limitations/Implications: Strain evaluations were limited to controlled greenhouse conditions.

Conclusion: The YM1, YM4 and YM5 strains show potential for further evaluation as biofertilizers for corn cultivation under field conditions.

Keywords: sustainable strategies, plant growth, biofertilization.

INTRODUCTION

Corn (*Zea mays* L.) is considered the most important cereal in the world (Kurtz *et al.*, 2016). In Mexico it is a basic crop for human and animal nutrition; it ranks first regard its acreage with approximately seven million hectares and a production volume of 23 million tons. Despite this, its national demand is around 39 million tons, so there is a production deficit (FAOSTAT, 2020; Reyes *et al.*, 2018). Given this situation, it is important to increase corn's

national production. However, this crop is highly extractive of the soil and therefore usually receives chemical fertilization, which represents 40-50% of their production cost and results in a lower profit margin for their producers (Reyes *et al.*, 2018). The periodic use of fertilizers affects the environment and human wellbeing (Olanrewaju and Babalola, 2019). For this reason, it is important to investigate environmentally friendly production alternatives. In this sense, the bacteria found in the region around the plant's root systems are also known as plant growth-promoting rhizobacteria (PGPR) can be a strategy (Olanrewaju and Babalola, 2019). These bacteria can promote plant growth through various mechanisms, such as biological nitrogen fixation, and phosphorus, potassium, and some micronutrients solubilization, as well as promoting phytohormones synthesis and other metabolites associated with pathogens biocontrol such as antibiotics and siderophores. (Olanrewaju *et al.*, 2017). There are several reports of groups of rhizobacteria associated with corn that promote its growth (Abedinzadeh *et al.*, 2019; Bjelić *et al.*, 2018; Karnwal, 2017; Richard *et al.*, 2018; Toribio-Jiménez *et al.*, 2017); however, native microorganisms may be better adapted to a specific region, making them ideal in strain selection processes, given that they could be more competitive than introduced bacteria (Karagöz, 2012). In this regard, even though in the state of Campeche, Mexico, corn is the main cultivated grain, with an area of approximately 150 thousand hectares (SAGARPA, 2019), there are no reports of native rhizobacteria used in its cultivation. Based on the above, the present work aims to isolate and determine in a greenhouse environment the biofertilizing potential of rhizospheric bacteria associated to corn (*Zea mays* L.) at Campeche, Mexico.

MATERIALS AND METHODS

The samples were collected from corn rhizospheric soil with different management conditions. At the ejido El Poste, Hopelchén, Campeche (19° 52' 12" N and 89° 52' 16" W) the samples considered as mechanized soil were taken, while at ejido Hool, Champotón, Campeche (19° 29' 84" N and 90° 26' 03" W) the non-mechanized soil samples. The rhizospheric soils were collected at a 0-20 cm depth, sampling five subsamples (golden five) that formed a composite sample. The soil was dried at room temperature for two days, sieved and stored in refrigeration until its microbiological analysis. The bacteria isolation was carried out via serial dilutions on base 10 following the methods by Velázquez-Gurrola *et al.* (2015). For this, 10 g of rhizospheric soil were diluted in 90 mL sterile water, up to 1/107. The specific solid media used were Pikovskaya and Rennie at 28 °C incubation. The different colonies were isolated and purified by exhaustion streak. The isolated strains were macroscopically characterized by polyphasic taxonomy and microscopically by Gram stain (Vincent and Humphrey, 1970). Likewise, the catalase test was performed (Hayward, 1960).

In vitro plant growth promotion was determined by inoculating 20 μ L of each isolated strain in different specific media. The growth of the strain in Rennie medium indicates its nitrogen-fixing ability (Rennie, 1981). The phosphorus solubilization was determined in Pikoskaya agar medium as described by Ramírez *et al.* (2014). Potassium solubilization was determined with a modified Pikoskaya medium following Velázquez-Gurrola *et al.* (2015).

Organic acid production was determined as described by Ogale *et al.* (2018). The phosphorus or potassium solubilization index (PSI or KSI) was calculated as the PSI or KSI ratio=(zone of halo+colony diameter)/colony diameter (Ramírez *et al.*, 2014.). The production of indole-3-acetic acid (IAA) was determined as described by Sarker and Al-Rashid (2013) using Salkowski reagent. The strains were grown in liquid Luria Bertani (LB) media and Nutritive Broth (NB) (given the bacteria requirement) with tryptophan (0.1%). The supernatant was used for IAA quantification with the aid of a Spectroquant NOVA60 spectrophotometer at a 540 nm length. The indole compound concentration was calculated with a linear regression equation of the calibration curve constructed with known IAA concentrations.

The *in vivo* growth promotion determination in corn plants was carried out in a greenhouse at the Campus Campeche of the Colegio de Postgraduados (19° 49' 79" N; 90° 54' 76" W). For it, the strains were selected and named as YM1, YM3, YM4, YM5 and for their effect in the *in vitro* tests. Bacteria were grown for five days in LB or CN media in an incubator (Thermo scientific MAXQ 4450) with shaking at 150 rpm at 28 °C. The cultures were centrifuged at 4000 rpm and the inoculants were prepared at a 10⁸ CFU mL⁻¹ concentration from the bacterial cell pellet. Corn seeds of the Dekalb 410 variety were disinfected with sodium hypochlorite (2%) and ethanol (96%) and placed in Petri dishes with sterile filter paper for 7 days until germination. Later, the plants were transplanted placing one plant per pot with a sterile substrate (earth, perlite and Peat

moss). After 3 d, the plants were inoculated with 1 mL of the bacteria. In total, six treatments were evaluated in a completely randomized experimental design with five repetitions, these corresponded to: T1) control (with no inoculation), T2) inoculation with the YM1 strain, T3) inoculation with the YM3 strain, T4) inoculation with the YM4 strain, 5) inoculation with strain YM5 and T6) inoculation with strain YM6. Thirty days after inoculation, the following variables were assessed: Plant height (PH), stem diameter (SD), root length (RL), stem fresh weight (SFW), stem dry weight (SDW), fresh root weight (FRW) and root dry weight (RDW). The data obtained in the *in vitro* and *in vivo* tests were analyzed in the SAS statistical software for Windows version 9, through an analysis of variance (ANOVA) and comparison test of means (LSD, $p \leq 0.01$) (Steel and Torrie, 1986).

RESULTS AND DISCUSSION

In total 16 rhizospheric bacteria were isolated from corn soil subjected to different management conditions. A higher number of bacteria was isolated from non-mechanized soil from the ejido of Hool, Champotón, Campeche ($n=10$). A lower number, from mechanized soil from the ejido Poste, Hopelchén, Campeche ($n=6$) (Table 1). This can be explained, excessive mechanization has been documented to affect soil quality and the development of beneficial microorganisms (Padron et al., 2012).

From the total isolates, 62.5% (10 strains) were Gram-positive, the remaining Gram-negative (Table 1). Similarly, Toribio-Jiménez et al. (2017) and Abedinzadeh et al. (2019) reported a higher number of Gram-positive in corn. The 56% of the isolated strains were aerobic or catalase-positive, similar to those reported by Karnwal (2017).

Regard the *in vitro* plant growth promoter potential of the isolated strains, a nitrogen-fixing bacteria predominance (81%) was observed (Table 1). These results concur with reports of rhizospheric soils from corn (Toribio-Jiménez et al., 2017; Karnwal, 2017, Richard et al., 2018). In the production of indoloactic acid (IAA), only 25% of the strains had this activity, the statistical analysis determined significant differences between them. The YRC2 strain reported the highest IAA production ($4.264 \mu\text{g mL}^{-1}$) registering statistical differences regard to the GRC4 ($1.739 \mu\text{g mL}^{-1}$), YM6 ($0.526 \mu\text{g mL}^{-1}$) and YM4 ($0.736 \mu\text{g mL}^{-1}$) strains. Olanrewaju and Babalola (2019) reported similar percentages in the number of bacteria capable of

producing IAA in corn (20%). Similarly, the IAA production values found in the bacteria in this study were similar to those previously reported in bacteria obtained from rhizospheric soil of corn (0.10 to $3.6 \mu\text{g mL}^{-1}$) (Mehnaz et al., 2010).

Respect phosphorus solubilization, 56% of the strains reported this activity. The statistical analysis of the phosphorus solubilization index (PSI) showed statistically significant differences between the strains. The GPA2 (0.453 mm) and YM1 (0.426 mm) strains had the highest ISP with no statistical difference between both. The YM6 (0.386 mm), YPB2 (0.363 mm) and YM3 (0.346 mm) strains had a medium activity PSI (Table 1). Olanrewaju and Babalola, (2019) reported a lower phosphorus solubilizing bacteria percentage in corn rhizospheric soil (29%), of which three reported medium and seven had slight activity.

Regard the potassium solubilization, 31% of the strains reported this capacity; however, no statistical significance was observed (Table 1). The main mechanism of potassium and phosphorus solubilization in bacteria is by organic and inorganic acids production (acidolysis) (Meena et al., 2014; Paredes-Mendoza and Espinosa-Victoria, 2010), in this study the high number of bacteria capable of producing organic acids (50%) suggests this is an important mechanism used by bacteria isolated from corn.

In the *in vivo* greenhouse plant growth promotion tests, statistical differences were observed in the assessed variables between the evaluated treatments. Plants treated with T4 (YM4) (63.33 cm) showed a higher PH (Table 2). This strain can produce IAA *in vitro*, as this auxin has regulatory effects on the growth and development of plants (Vessey, 2003), it could explain its effect on the PH variable. In turn, T5 treatment (YM5) reports the highest SD (7.13 mm), RL (36.78 cm) and SFW (12.03 g per plant). Also, the T2 (YM1) treatment showed the highest RDW (20.00 g per plant) and RDW (2.01 g per plant) (Table 2).

The data obtained on the root and shoot dry weight were higher than those previously reported from two corn varieties due to the application of *Pseudomonas putida* (CR7) and *Sphingobacterium canadense* CR11 bacteria (Mehnaz et al., 2010). The YM1 and YM5 strains *in vitro* showed nitrogen fixation ability, organic acids production, and phosphorus and potassium solubilization. In this regard, it has been reported that nitrogen promotes rapid

Table 1. Biochemical and physiological properties involved in plant growth-promoting of bacterial strains isolated from two sites at the state of Campeche, Mexico.

Site	strain	Gram reaction	Catalase test	Solubilization				Fixation of N	HCL	IAA($\mu\text{g mL}^{-1}$)
				P	PSI (mm)	K	KSI (mm)			
The ejido El Poste, Hopelchén	YM1	+	-	+++	0.426 \pm 0.02ab	++	0.475 \pm 0.14a	+	+	-
	YM2	-	-	-	-	-	-	+	+	-
	YM6	-	+	++	0.386 \pm 0.02 bc	++	0.566 \pm 0.09a	-	-	0.526 \pm 0.07c
	GRC4	-	-	-	-	-	-	+	-	1.739 \pm 0.10b
	GRB4	+	+	-	-	-	-	+	+	ND
	GPA2	+	-	+++	0.453 \pm 0.004 a	++	0.544 \pm 0.05a	-	-	-
The ejido Hool, Champotón	YRB6	+	+	-	-	-	-	+	+	-
	YPB2	+	+	++	0.363 \pm 0.00cd	++	0.483 \pm 0.04a	-	-	-
	YRA5	-	+	++	0.336 \pm 0.01de	-	-	+	+	-
	YRC4	+	-	+	0.166 \pm 0.08f	-	-	+	+	-
	YRB2	-	+	-	-	-	-	+	-	ND
	YRC2	+	+	-	-	-	-	+	-	4.264 \pm 0.24a
	YM3	+	-	++	0.346 \pm 0.0 cde	++	0.530 \pm 0.06a	+	-	-
	YM4	-	+	-	-	-	-	+	-	0.736 \pm 0.10c
YM5	+	-	++	0.313 \pm 0.05 e	-	-	+	+	-	
YRB7	+	+	+	0.140 \pm 0.01 f	-	-	+	+	-	

+: positive activity, -: negative activity, +++: high production, ++: medium production, +: low production; PSI = phosphorus solubilization index, KSI = potassium solubilization index. HCL: production of organic acids, ND: not determined. IAA: production of indoleacetic acid. Means with same letters within each column are not statistically different (LSD, 0.05).

Table 2. Effect of the inoculation of the selected strains on the growth in corn plants after 30 days of the inoculation.

Treatments	PH (cm)	SD (mm)	RL (cm)	SFW (g plant^{-1})	SDW (g plant^{-1})	RFW (g plant^{-1})	RDW (g plant^{-1})
T1: with no inoculation	59.57 \pm 2.3b	6.57 \pm 0.2ab	27.17 \pm 1.4d	8.37 \pm 0.1 b	1.97 \pm 0.2 a	13.14 \pm 2.1c	1.61 \pm 0.1c
T2:YM1	61.15 \pm 0.8 ab	6.63 \pm 1.1ab	33.40 \pm 1.2ab	11.12 \pm 2.3ab	1.63 \pm 0.3ab	20.00 \pm 0.0 a	2.01 \pm 0.1a
T3: YM3	62.47 \pm 2.1 ab	6.70 \pm 0.5ab	31.57 \pm 0.9bc	12.00 \pm 0.9a	1.84 \pm 0.1ab	17.15 \pm 0.6b	1.87 \pm 0.1ab
T4: YM4	63.33 \pm 4.1a	6.70 \pm 0.3ab	30.50 \pm 1.0bcd	10.92 \pm 2.4ab	1.68 \pm 0.4ab	17.57 \pm 2.8ab	1.71 \pm 0.2bc
T5: YM5	60.90 \pm 1.0ab	7.13 \pm 1.0 a	36.78 \pm 4.3a	12.03 \pm 3.8a	1.90 \pm 0.6ab	17.12 \pm 1.3 b	1.81 \pm 0.2ab
T6: YM6	59.37 \pm 0.3b	5.80 \pm 0.9b	28.67 \pm 2.0cd	11.60 \pm 0.7ab	1.30 \pm 0.1b	10.98 \pm 0.8 c	1.27 \pm 0.1d

PH: Plant height, SD = stem diameter; RL = root length; SFW = stem fresh weight; SDW: stem dry weight, RFW: root fresh weight, RDW = root dry weight. Means (n = 10) with the same letters in each column are not statistically different (LSD, 0.05). \pm = standard deviation.

cell division and elongation (Peña and Reyez, 2007), coupled with the fact that phosphorus is an important micronutrient for plant growth since it participates in multiple metabolic processes (Karnwal, 2017). Viruel *et al.* (2014) demonstrated that phosphorus solubilizing bacteria can stimulate stem growth and higher biomass production.

The results indicate that the YM1, YM4 and YM5 rhizobacteria have positive effects on growth promotion

of corn plants and therefore have great potential to be used in the field as biofertilizers in this study region.

CONCLUSION

Sixteen bacteria strains were isolated and a greater number of strains from non-mechanized soils. The plant growth promoter activity with the highest percentage of bacteria corresponded to nitrogen fixation. The lowest activity corresponded to IAA production. YM1, YM4 and YM5 strains showed a positive effect in promoting plant

growth of corn plants in *in vivo* tests. Therefore, they show the potential to be evaluated as biofertilizers for corn cultivation under field conditions.

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