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Short communication

Effect of the inoculation of PGPR isolated from corn on the growth of this crop under controlled conditions

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ABSTRACT

The application of inoculants formulated based on plant growth promoting rhizobacteria in crops of agricultural interest such as corn, represents an ecological alternative to the use of chemicals in agriculture. On the other hand, the use of native strains for the inoculation of plants could represent an advantage over the use of alien strains and, therefore, improve crop production. Consequently, the present work aimed to evaluate the inoculation effect of 20 bacterial strains, isolated from the rhizosphere of corn and previously characterized, corresponding to the genera *Stenotrophomonas*, *Pseudomonas*, *Rhizobium* and *Enterobacter*, in the development of morphoagronomic variables of its own culture. For this, inoculants were prepared with each of the strains in liquid LB medium. The inoculation was carried out on the corn seeds sown in unsterilized Red Ferrallitic soil, at a rate of 300 µl of inoculum per seed. The experiment was established under controlled conditions of light, relative humidity, temperature and irrigation and it was determined: plant height, root length, aerial dry mass, root dry mass and concentration of total chlorophylls, 30 days after inoculation. The best results were obtained with treatments inoculated with *Stenotrophomonas* sp. INCA-FRr1, *Stenotrophomonas* sp. INCA-FRc24 and *Rhizobium* sp. INCA-FRc1. This study represents the basis for the conception of a new bioproduct destined for the fertilization of corn crop.

Key words: FBN, *Stenotrophomonas*, *Rhizobium*, nutrition, phytostimulation

INTRODUCTION

The soil houses a large number of living beings, mainly microorganisms. The genetic variety and the diversity of ecological niches of microbial populations have a high impact on soil functions and, especially, on plant growth and development [(1)]. Interactions established between plant roots and edaphic microorganisms constitute a dynamic environment called rhizosphere [(2)], where the diversity and size of microbial populations is higher compared to uncultivated soil [(3)]. These populations actively participate in the biogeochemical cycles of nutrients, mainly nitrogen and phosphorus, produce plant hormones, synthesize antibiotics, among other characteristics and, as a result, favor the establishment, nutrition and development of plants [(4)].

Corn is one of the most important cereals, from a nutritional point of view; also, it is the most cultivated and harvested worldwide, along with wheat and rice [(5)]. Given the domestication process that this crop has undergone, it is necessary to use large amounts of fertilizers to obtain acceptable yields [(6)]. Fertilization, especially the incorporation of mineral nitrogen to the crop, represents the highest cost of the production process [(7)]; Furthermore, the irrational use of these inputs has a negative impact on the agroecosystem [(8)] and even on human health [(9)].

The manufacture and application of inoculants formulated with various microbial species is a well-known practice in agriculture. Currently, within the framework of sustainability, the search for new microorganisms with various properties that promote plant growth is an emerging line of research, since in certain circumstances, they can partially replace the use of pesticides and chemical fertilizers [(10)]. The use of native strains as inoculants promote the ecological-sustainable management of agroecosystems and could improve crop production [(11)]. The ability of native strains to interact positively with the resident edaphic microbiota and their adaptability to local climatic and agroecological conditions often enhances their performance compared to non-native strains [(12)].

The objective of this work was to evaluate the inoculation effect of 20 strains, characterized as PGPR, isolated from the corn rhizosphere, corresponding to four bacterial genera (*Stenotrophomonas*, *Pseudomonas*, *Rhizobium* and *Enterobacter*), on the growth of low corn plants. controlled conditions.

MATERIALS AND METHODS

Microbiological material

Twenty strains isolated from the rhizosphere of commercial corn cultivars 'Raúl' and 'Canilla' were used. These were previously identified and were characterized, based on their ability to

perform BNF, solubilize phosphorus and potassium salts and inhibit mycelial growth of the pathogen *Fusarium oxysporum* [13]. The characteristics of the strains are shown in Table 1.

Table 1. Identification and qualitative characterization as PGPR of 20 strains from the rhizosphere of *Zea mays* L. cultivars 'Raúl' and 'Canilla'

Strains	Identification	BNF	Solubilization (PO_4^{-2})	Solubilization (K^+)	Antagonism
INCA-FRr1	*Stenotrophomonas* sp.	+	+	+	+
INCA-FRr2	*Pseudomonas* sp.	+	+	-	+
INCA-FRr3	*Pseudomonas* sp.	+	+	-	+
INCA-FRr4	*Pseudomonas* sp.	+	+	-	-
INCA-FRr5	*Pseudomonas* sp.	+	+	+	-
INCA-FRr6	*Stenotrophomonas* sp.	+	+	-	-
INCA-FRr7	*Stenotrophomonas* sp.	+	+	-	+
INCA-FRr8	*Pseudomonas* sp.	+	+	-	+
INCA-FRr9	*Pseudomonas* sp.	+	+	-	-
INCA-FRr10	*Rhizobium* sp.	+	-	-	-
INCA-FRr11	*Pseudomonas* sp.	+	+	-	-
INCA-FRr12	*Stenotrophomonas* sp.	+	+	-	-
INCA-FRr13	*Stenotrophomonas* sp.	+	+	-	-
INCA-FRr16	*Enterobacter* sp.	+	+	-	-
INCA-FRc1	*Rhizobium* sp.	+	-	-	-
INCA-FRc4	*Rhizobium* sp.	+	-	-	-
INCA-FRc8	*Rhizobium* sp.	+	-	-	-
INCA-FRc16	*Stenotrophomonas* sp.	+	+	-	-
INCA-FRc19	*Rhizobium* sp.	+	-	-	-
INCA-FRc24	*Stenotrophomonas* sp.	+	+	-	+

Preparation of inoculants

Inoculants were prepared in Erlenmeyer flasks of 150 mL capacity, with 20 mL of liquid LB medium. These were inoculated with a roast of each strain, stored at 4 ºC in solid LB medium. The flasks were kept under shaking conditions on a thermostated orbital shaker at 150 rpm and 29 °C for 24 h. The optical density was adjusted by spectrophotometry to 0.5 (λ = 600 nm) in each inoculant.

Inoculation in corn plants under controlled conditions

Corn cultivar ‘Raúl’ seeds were used, from the seed bank of the Department of Genetics and Plant Breeding of the National Institute of Agricultural Sciences (INCA). These were superficially disinfected with 70 % ethanol for 5 min, 20 % sodium hypochlorite, for 10 min

and six consecutive washes with sterile distilled water. Then, they were placed superficially in pots with 700 g of Leached Red Ferralitic soil, not sterilized, at the rate of two seeds per pot. The inoculation was carried out with 300 µl of inoculum on each seed, establishing 20 treatments and a non-inoculated control. Five days after inoculation, the less developed or non-germinated seedling was extracted from each pot, keeping one plant per pot. The test was maintained for 30 days under controlled conditions of photoperiod (12 h light/12 h darkness), temperature (day/night 26/22 °C) and relative humidity (70 %). Irrigation was carried out every three days for all treatments, including the control, with a modification of the Hoagland nutrient solution (5 gL^{-1} KH_2PO_4, 27 g L^{-1} $MgSO_4.7H_2O$, 0.14 g L^{-1} H_3BO_3, 0.15 g L^{-1} $CuSO_4.5H_2O$, 0.008 g L^{-1} $(NH_4)_6Mo_7O_{24}.6H_2O$, 0.06 g L^{-1} $ZnSO_4.7H_2O$, 0.2 g L^{-1} $MnSO_4.4H_2O$, 1.87 g L^{-1} Fe-EDTA (6%)), from which the nitrogenous salts were removed. Subsequently, height (cm), root length (cm), aerial dry mass (ADM) (g), radical dry mass (RDM) (g) and total chlorophyll content (SPAD)

Diseño y análisis estadístico Statistical design and analysis

Eight replications per treatment were established and a completely randomized design was used. Variable values that were determined were subjected to the Bartlett normality test and the Kormogorov-Smirnov homogeneity of variance test. Subsequently, a simple classification analysis of variance was applied, using the Tukey mean comparison test for $p < 0.05$. The SPSS Statistic program (ver. 21) was used for the statistical processing of the data. The experiment was carried out in triplicate.

RESULTS AND DISCUSSION

Inoculation in corn plants under controlled conditions

At 30 days after inoculation, statistical differences were observed between the growth indicators evaluated in each of the treatments (Table 2).

The treatments with the best results corresponded to the plants inoculated with the INCA-FRr1 and INCA-FRc24 strains, identified as *Stenotrophomonas*, in addition to INCA-FRc1, identified as *Rhizobium*. These treatments presented statistically superior values to the control in four of the five variables evaluated, which could be related to the mechanisms of plant growth promotion that they present.

Table 2. Inoculation effect of bacterial strains on the growth of corn plants cultivar 'Raúl', under controlled conditions

Strains	Height (cm)	Root Length (cm)	ADM (g)	RDM (g)	Total chlorophylls (SPAD)
INCA-FRr1	65.34 a	38.60 ab	0.63 abc	0.53 a	19.14 d
INCA-FRr2	57.90 abcd	35.79 abc	0.70 a	0.45 abc	28.56 abc
INCA-FRr3	61.64 abc	35.04 abc	0.66 ab	0.42 abc	25.96 bc
INCA-FRr4	59.52 abcd	35.82 abc	0.55 adcd	0.36 abc	27.59 abc
INCA-FRr5	58.26 abcd	34.40 abc	0.60 abcde	0.43 abc	29.26 abc
INCA-FRr6	53.40 de	38.34 ab	0.51 bcde	0.40 abc	33.38 a
INCA-FRr7	55.32 bcd	41.98 a	0.58 abcd	0.51 a	23.94 cd
INCA-FRr8	52.14 de	34.38 abc	0.49 bcde	0.41 abc	26.75 bc
INCA-FRr9	63.76 ab	30.60 c	0.64 abc	0.49 a	19.35 d
INCA-FRr10	56.04 abcd	32.70 bc	0.55 abcd	0.53 a	26.44 bc
INCA-FRr11	57.30 abcd	32.00 bc	0.48 cde	0.44 abc	28.43 abc
INCA-FRr12	60.04 abcd	35.04 abc	0.52 abcde	0.48 ab	28.01 abc
INCA-FRr13	54.08 cde	37.90 ab	0.50 bcde	0.38 abc	28.86 abc
INCA-FRr16	61.53abc	35.06 abc	0.67 ab	0.44 abc	24.22 cd
INCA-FRc1	64.90 a	37.20 ab	0.66 abc	0.50 a	18.74 d
INCA-FRc4	57.24 abcd	37.92 ab	0.56 abcd	0.40 abc	23.25 cd
INCA-FRc8	44.66 f	34.80 abc	0.34 f	0.36 abc	33.83 a
INCA-FRc16	64.80 a	37.18 ab	0.52 bcde	0.39 abc	29.74 abc
INCA-FRc19	53.40 de	30.90 bc	0.44 de	0.28 c	29.08 abc
INCA-FRc24	43.89 f	37.84 ab	0.63 abc	0.52 a	33.66 a
Control	51.24 e	29.60 c	0.39 ef	0.31 bc	18.70 d
Standard error	0.794	0.593	0.012	0.015	1.793

The mean of each treatment and the standard error for each measured variable are represented.

Equal letters in the same column do not differ significantly (Tukey $p < 0.05$, $n = 8$)

Treatments with the strains Pseudomonas sp. INCA-FRr9 *Stenotrophomonas* sp. INCA-FRr12 and *Stenotrophomonas* sp. INCA-FRc16. The genera *Stenotrophomonas*, *Pseudomonas* and *Rhizobium*, are reported as natural, rhizospheric and endophytic microbiota of various crops including corn [14–16]. Previous works ensure the phytostimulant effect of these microorganisms on different crops of agricultural interest, both legumes and non-legumes [17-20].

Plant height does not tend to increase much more than the non-inoculated controls, after applying some biological treatment [21]; however, the results show significant increases of up to 27.5 % between the INCA-FRr1, INCA-FRc1 and INCA-FRc16 strains, with respect to the non-inoculated control. Different tests of inoculation of *Rhizobium* and *Stenotrophomonas* in corn result in a slight increase in the length of the plants, in comparison with the results obtained in this investigation [21–24]. On the other hand, the root length was favored in the plants

inoculated with these same treatments, in addition to the INCA-FRc24 and INCA-FRr7 strains. According to the literature, inoculation with different species of *Rhizobium* enhances an increase in root length, root number and root dry mass in corn plants [22]. In the case of inoculation with *Stenotrophomonas*, significant increases in root development have not been reported in previous research [24–26]; however, the best results in this trial correspond to treatment with *Stenotrophomonas* sp. INCA-FRr7, which presented an increase in root length of 41.8 %, above the control treatment.

The strains that contributed the most to the development of the aerial dry mass were *Pseudomonas* sp. INCA-FRr2, *Pseudomonas* sp. INCA-FRr3 and *Enterobacter* sp. INCA-FRr16, which presented increases of up to 79.5% compared to the control. Similar results are reported in the literature for some species of *Enterobacter* and *Pseudomonas*, which are mainly attributed to BNF and phytohormone production [27,28]. Regarding the radical dry mass, increases of up to 71 % were obtained, with respect to control plants in the INCA-FRr1, INCA-FRc1 and INCA-FRc24 treatments, mainly. Some authors suggest that the growth and development of morphoagronomic variables in corn is caused by a sum of factors and not by individual values obtained *in vitro* [29]. Others affirm that the products from BNF contribute in high percentages to the total biomass development of maize and highlight the role of auxins as the main causes of the increase in root and aerial biomass [30].

On the other hand, the highest concentrations of total chlorophylls were obtained, again, in treatments inoculated with *Rhizobium* and *Stenotrophomonas*, highlighting the INCA-FRr6, INCA-FRc8 and INCA-FRc24 strains, with an increase of 81 %, above the control. The chlorophyll content in plant is closely related to its nutritional status [31] and, particularly, to the nitrogen content as an essential component of this biomolecule [32]. Various investigations estimate a direct relationship between the production of chlorophylls and the supply of nitrogen to the plant, based on obtaining higher concentrations of total chlorophylls, as the dose of mineral nitrogen in the soil increases [32,33]. Thus, one of the important factors that indicates the efficiency of nitrogen fertilization is the content of photosynthetic pigments in leaves, since the proteins of the Calvin cycle and of the thylakoids represent most of the foliar nitrogen [21].

Taking into account that all the strains used had the ability to perform BNF, it could be said that this aspect favored the production of total chlorophylls. However, plants inoculated with the INCA-FRr1 and INCA-FRc1 strains showed the lowest values of this variable, which contrasts with the rest of the variables, in which both treatments stood out positively. This could suggest that perhaps, BNF was not the main plant growth promotion mechanism used by these strains.

In this research, the soil used came from the same region from which the inoculated strains were isolated; furthermore, it was not sterilized for the trial, so there should be no significant changes in the resident microbiota [34]. Indeed, the microbial populations present in this soil must have influenced the plant growth promoting activity of the inoculated strains, either by enhancing or inhibiting it. Therefore, it could appreciate that there is compatibility and synergism between the INCA-FRr1, INCA-FRc1 and INCA-FRc24 strains and the resident edaphic microbiota. On the other hand, mineral fertilizer was not applied to the treatments, which could influence the expression of plant growth promotion mechanisms presented by inoculated strains, especially BNF.

It has been shown that a deficiency in nitrogen compounds in the medium stimulates the synthesis of the enzymatic complex Nitrogenase, responsible for BNF [35]. In this case, the low content of organic matter present in the Red Ferrallitic soils [36], the nitrogen elimination of compounds from the Hoagland nutrient solution and the lack of mineral fertilizer, could enhance conditions for the expression of nitrogen-fixing activity of these strains. On the other hand, and without ruling out the fundamental role of this element in the growth of plants, especially corn, it is possible that these microorganisms present other mechanisms to promote plant growth that have not been determined in this work. It should also be noted that the genera *Stenotrophomonas* and *Rhizobium*, the most outstanding in this trial, despite their diazotrophic character, in non-legume plants are better known for their phytostimulant capacity than for the contribution of BNF [21–24,37,38]. For these reasons, it would be convenient to carry out a more complete characterization of the strains studied.

CONCLUSIONS

- The inoculants made up of *Stenotrophomonas* sp. INCA-FRr1, *Stenotrophomonas* sp. INCA-FRc24 and *Rhizobium* sp. INCA-FRc1, presented significant increases with respect to the non-inoculated control in a greater number of variables. Although increasing nitrogen availability through BNF is essential for plant growth, there could be other mechanisms that contribute to it significantly. For this reason, it is necessary to delve into other characteristics that promote plant growth that these strains could present.
- The INCA-FRr1, INCA-FRc1 and INCA-FRc24 strains are promising inoculants for a new phase of experimentation under semi-controlled and field conditions.

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