Investigation note

Actinobacteria with antagonistic potential *in vitro* to phytopathogenic fungi and promotion of growth in chili plants

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Abstract

The inhibitory potential of three strains of actinobacteria (B21, B22 and B37) against phytopathogens: *Rhizoctonia solani*, *Phytophthora capsici* and *Fusarium oxysporum* was evaluated *in vitro* using the technique of aperados culture, where at 72 h after confrontation the percentage of radial growth inhibition (PICR) by actinobacteria was evaluated. The results showed a variable PICR between 67.54 and 93.84% depending on the pathogen. Strain B22 was the one that showed an average PICR of 98.73% for the three phytopathogens. In the *in vivo* scrutiny, the chili plants inoculated with the actinobacteria showed higher plant height and greater dry weight of the fruits with respect to the control, which suggests that actinobacteria promote the growth of chili plants.

Keywords: *Rhizobacteria*, fungal inhibition, growth promotion.

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In Mexico, the diseases of agricultural crops, caused by fungi and bacteria are mainly controlled by means of chemical compounds. In 2015, 28 741.46 t of active ingredient of fungicides and bactericides were used, all these compounds were applied on an area that amounts to more than 22 million hectares cultivated in the country (FAO, 2019).

The use of synthetic pesticides in agriculture has caused negative effects on human health, contamination of water tables, high production costs, in addition to acting negatively on beneficial organisms, this under poor control derived incorrect applications in concentration and frequency, affecting the environment, causing an imbalance in the ecosystem and with it, loss of biodiversity (Gutiérrez-Ramírez *et al*., 2013).

Biological control has proven to be an effective and economically viable tool for the control of pests and diseases, in which natural enemies, antagonists or living competitors are employed, capable of maintaining the population density of a pest organism at a level that does not cause economically important damages and maintaining the sustainability of agroecosystems (Infante *et al*., 2009; Gutiérrez-Ramírez *et al*., 2013).

Among the agents that perform biocontrol processes, are bacteria, fungi, viruses, predators and parasitoids, which have different mechanisms of action highlighting: the antibiosis, competition for space and nutrients or ecological niche, production of inhibitory compounds, activation of enzymes of the pathogenic agent, parasitism and the induction of resistance in the plant (Villamil *et al*., 2015; Reyes *et al*., 2015).

Actinobacteria are known to fulfill different roles in the ecosystem, such as decomposers of organic matter, especially biopolymers such as lignocellulose, starch and chitin, improve the structure of the soil and the production of bioactive compounds with antagonistic activity against pathogenic microorganisms; they can be found in the soil and root of plants, as well as in freshwater and saltwater sediments, they have the ability to biosynthesize a wide variety of antibiotics as secondary metabolites or natural products, and activities have also been described that can catalog actinobacteria as plant growth promoting rhizobacteria Plant Growth Promoting Rhizobacteria (PGPR) (Doubou *et al*., 2001; Franco-Correa, 2009).

Currently in Mexico the use of more environmentally friendly products is being implemented, which is why different niches are being explored in order to obtain strains of microorganisms with potential for the control of pests and diseases in economically important crops. One of these crops is the chili (*Capsicum annuum* L.) which is reduced its production due to the attack of the phytopathogenic fungi *Rhizoctonia solani*, *Phytophthora capsici* and *Fusarium oxysporum*, which cause damage to the root system and could not be controlled effective way.

The objective of this work was to isolate actinobacteria from disturbed and undisturbed soils and to demonstrate their potential in the inhibition of phytopathogenic fungi of *R. solani*, *P. capsici* and *F. oxysporum in vitro* conditions, as well as to evaluate the promotion of the growth of chili plants.

To address this objective, soil samples were taken in November and December 2016 from cultivated and uncultivated (undisturbed) soil in the municipalities of Apaseo el Grande, Celaya, Comonfort, Penjamo, San Luis de la Paz, San Diego of the Union and Tarimoro and in January, February and March of 2017 in the municipalities of San Miguel de Allende, Villagran and Xichu, all of the state of Guanajuato.

The collected soil samples were screened to obtain a homogeneous sample. One gram of soil from each sample was placed in 15 mL tubes, which contained 9 mL of sterile distilled H_2O and were processed by serial dilution (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}), were taken 50 µL of each of the dilutions and were placed in the center of Petri dishes with PDA (Papa Dextrose Agar) dispersing the dilution with sterile glass rod.

The number of Petri dishes inoculated was two Petri dishes for the 10^{-1} dilution, three Petri dishes; for dilution 10^{-2} and seven Petri dishes for dilutions 10^{-3} and 10^{-4}, all under aseptic conditions Rodríguez-Guerra, Com. Pers. (2017).

The inoculated Petri dishes were incubated at 26 °C until the growth of microorganisms was observed. When observing growth of colonies that suggested to be actinobacterias (phenotype with powdery texture, color, shape, surface and edge of the colony, as well as pigmentation of the culture medium and being Gram positive), the entire colony was taken with the help of a previously sterilized dissection needle and placed in Petri dishes with PDA.

In vitro scrutinies were performed with the purpose of observing the antagonistic potential of actinobacteria. Fragments of actinobacteria colony were rubbed in the center of 90 x 15 mm Petri dishes with PDA culture medium, covering a radius of approximately 3 cm from the central surface of the Petri dish. The inoculated Petri dishes were kept at 26 °C for 9 days, then 7 mm colony diameter explants were placed with 72 h of growth of the phytopathogenic fungi: *R. solani*, *P. capsici* or *Fusarium oxysporum*, at approximately 2 cm of the margin of the actinobacteria colony.

As a control, colony fragments of the same diameter of the phytopathogens without actinobacteria were placed in Petri dish with PDA in a similar way that in the comparison, they were also used as a control Petri dishes with PDA inoculated in the center only with actinobacteria, 3 replicas were made by actinomycete and phytopathogen.

The evaluation was made at 24, 48 and 72 h after the confrontation, where the radial growth of the mycelium of the confronted phytopathogens and of the controls was measured and the percentage of inhibition of radial growth (PICR) was determined using the formula of Ezziyyani *et al.* (2004), $PICR = (R1-R2)/R1 * 100$, where R1 is the radius of growth of the colony of the control pathogen and R2 is the radius of the colony of the pathogen in confrontation.

With the strains of actinobacteria that inhibited the growth of the fungi, a scrutiny was carried out to define their influence on the growth of chili plants. Each actinobacteria strain was grown in modified Spezieller Nahrstoffmmarmer Agar (SNA) culture medium (1 g L^{-1} KH_2PO_4, 1 g L^{-1} KNO_3, 0.5 g L^{-1} $MgSO_4$ $7H_2O$, 0.5 g L^{-1} KCl, 0.2 g L^{-1} sucrose, 0.2 g L^{-1} glucose and 5 g L^{-1} yeast extract) (Nirenberg, 1976). The culture medium was placed in an orbital shaker at 130 rpm and 28 °C for 13 days. Chili plants var. Santa Fe six weeks were inoculated with the potential actinobacteria.

The design of the experiment was completely randomized with three repetitions and five plants per repetition. At the time of the transplant and ten days after the transplant, 5 mL of actinobacteria spore suspension was added to each plant at a concentration of $1*10^7$ CFU mL^{-1} and in the control plants only 5 mL of sterile water was added at neck level. The plants were evaluated in the production stage and the variables evaluated were plant height, root length, fruit length and fresh fruit weight.

Eighty-two strains of actinobacteria were identified from the sampled localities, where the highest number of strains was obtained from the municipality of San Miguel de Allende (undisturbed soil) with 60 strains, followed by Celaya with 10 strains. Of the 82 strains of actinobacteria confronted with the phytopathogens, three strains of them showed inhibitory potential against *R. solani*, *P. capsici* and *F. oxysporum*. The potential actinobacteria strains were: B21 and B22 (Sierra de Xichu, undisturbed area) and B37 (Pénjamo, of a farm cultivated with corn).

The results of the comparison showed a range of PICR from 67.54 to 93.84% (Table 1), depending on the pathogenic fungus. It should be noted that the inhibition of phytopathogens by the actinomycetes occurred without any contact between them, which is why the presence of metabolites secreted and expanded in the culture medium is suggested (Franco-Correa, 2009; Rodríguez-Villarreal *et al*., 2014).

Table 1. Percentage of inhibition of radial growth of *Rhizoctonia solani*, *Phytophthora capsici* and *Fusarium oxysporum* by actinobacteria from the state of Guanajuato.

Actinobacteria	Pathogens			Average (%)*
	R. solani	*P. capsici*	*F. oxysporum*	
B21	89.81	44.74	54.1	62.88
B22	96.18	100	100	98.73
B37	95.54	57.89	97.54	83.66
Average %	93.84	67.54	83.88	81.76

*= Average percentage obtained with the formula of Ezziyani *et al.* (2004).

It should be noted that the strain B22 showed consistency in the percentage of inhibition to the three chili pathogens, while B21 showed a reduced capacity of inhibition for the three pathogens. The pathogen that presented the highest PICR for the three strains of actinobacteria was *R. solani* with an average value of 93.84%, while *P. capsici* presented lower PICR for all strains of actinobacteria with 67.54% (Table 1).

According to Medina *et al*. (2014) the average PICR obtained for *P. infestans* was 77.65 and 75.33% with the two best actinomycete strains of 14 strains evaluated; while for *R. solani* the average PICR they obtained was 100 and 83.83%, respectively, results similar to those obtained in this trial (PICR 93.84%).

On the other hand, although strains B21 and B22 belong to the same municipality, they also differ in the antagonistic potential, particularly for the control of *P. capsici* and *F. oxysporum* (Table 1), according to Anwar *et al*. (2016), actinobacteria have different mechanisms of action in the inhibition of phytopathogenic agents, since they produce siderophores and antibiotic and fungicide substances differentially, even when the strains belong to the same species and to the same ecological niche.

The results obtained from the inoculation of the actinobacteria in chili plants showed that the height of the plant and the fresh weight of fruits were the variables that increased their values with the three strains with respect to the values of the control plants (Figure 1). The length of root and length of fruits did not show statistically significant effects with respect to the control for any of the strains of actinobacteria (Figure 1).

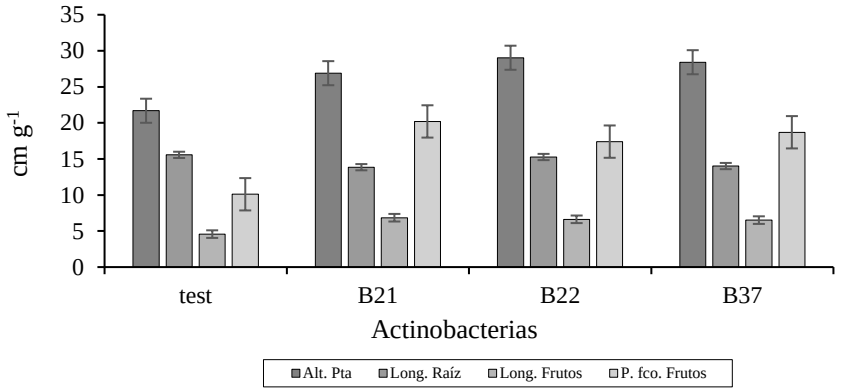

Figure 1. Effect of the application of actinobacteria on the variables. Alt. Pta= plant height; long. root= root length; long. fruits= length of fruits and p. fco fruits= fresh weight of fruits in chili plants.

Quantitatively speaking strain B22 showed greater effect in the four variables evaluated in chili plants than the other two strains, establishing a correlation with the effect of higher PICR for the three pathogens evaluated; however, the type of metabolites that promote both plant growth and inhibition of pathogens is still to be defined.

The results obtained in the evaluated variables of the chili plants are attributed to the fact that the actinobacteria served as growth promoters, probably inducing the increase in the production of indole acetic acid (AIA), auxin that naturally produces the plant, showing an effect in the growth of stem thickness and length, as well as in fruits, in addition to gibberellins and substances similar to cytokinins, favoring the development of plants and the stimulation of the development of lateral roots and root hairs (Palaniyandi *et al*., 2013).

Conclusions

The three strains of actinobacteria showed a high inhibitory potential against *R. solani*, *P. capsici* and *F. oxysporum* at the *in vitro* level, which suggests carrying out scrutinies at the plant level and evaluating if the biocontrol effect is maintained. At the plant level it was observed that the application of actinobacteria promotes the growth of the plant with respect to the control. It is desirable to establish the differential of metabolites that act and the mode of action in both analyzes to define the best combination in disease management trials.

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