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RESEARCH ARTICLE

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Characterization of the antifungal activity of three rhizobacterial strains against *Rhizoctonia solani*

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Abstract

In this study, three rhizobacterial strains were characterized by their ability to inhibit *Rhizoctonia solani* and tested in chili (*Capsicum annuum* L.) seedlings. Strains A46 and P61 were identified as *Pseudomonas tolaasii*, and S108 as *Rhanella aquatilis*. In the dual culture tests, all the strains inhibited the radial growth of *R. solani*. None of the three strains produced chitinases or volatile compounds, but they were found to produce siderophores. However, this last characteristic was not responsible for the rhizobacterial inhibitory effect on the growth of *R. solani*, other bacterial metabolites were possibly involved. The bacterial filtrates added to the potato dextrose agar medium (PDA) in a 3:7 ratio had significant antifungal activity, being the filtrate from strain S108 the one that showed the highest effect, with 56% fungal inhibition. The co-culture of strain A46 with the phytopathogenic fungi in potato dextrose broth (PDB) increased the antifungal activity of the rhizobacterial filtrate. The application of the rhizobacterial strains to Serrano chili decreased the *R. solani*-related mortality rate in seedlings; particularly, S108 had the greatest effect, which was similar to the fungicide effect. This study showed that the S108 strain has potential as a biofungicide to control *R. solani* in chili seedlings.

Additional keywords: antagonism; biopesticides; PGPR; fungal inhibition; bacterial metabolites.

Abbreviations used: CAS (chrome azurol S agar); PDA (potato dextrose agar); PDB (potato dextrose broth); R (radius of the fungus in the control); r (radius of the fungus in each treatment).

Authors´ contributions: Designed the work; interpreted the results, and wrote the manuscript: JJA. Molecular identification of rhizobacteria strains: DYPM, AGM. Performed the experiments, analyzed the data, and wrote the manuscript: DYPM. Analyzed the data and revised the manuscript: MPRG, OGB, and RAD.

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Introduction

Several genera of soil fungi are pathogens that cause severe damage to crops around the world, resulting in large yield losses (Abuzar, 2013; Naing *et al.*, 2014). One such soil-borne fungal pathogen is *Rhizoctonia*, which is widely distributed and causes diseases such as stem and root rot, damping-off, wilting, and leaf blight (Cao *et al.*, 2004). In chili peppers (*Capsicum annuum* L.), one of the most important spice crops in the world (Smith, 2015), *Rhizoctonia solani*, along with *Phytophthora capsici* and *Fusarium oxysporum*, cause severe damage to the roots and stems which negatively impacts on plant growth and crop yield (Velásquez-Valle *et al.*, 2001; Guillén-Cruz *et al.*, 2006; Lee *et al.*, 2008; Youssef *et al.*, 2016). Mexico is one of the largest exporters of chili peppers worldwide, and one of the challenges it faces is to reduce yield losses caused by root pathogens. This can be achieved through the use of different control strategies and one of them is to apply antagonistic microorganisms (Mojica *et al.*, 2009; Abuzar, 2013).

In recent years, there has been increasing interest in biological control as an alternative to the use of agrochemicals that have a negative impact on the environment (Huang *et al.*, 2012; Czaja *et al.*, 2015). The use of plant growth-promoting rhizobacteria (PGPR) can be a good option because, in addition to positively

affecting crops, several genera are natural antagonists of phytopathogenic fungi (Commare *et al.*, 2002; Saravanakumar *et al.*, 2007; Pliego *et al.*, 2011; Huang *et al.*, 2012). Biocontrol through rhizobacteria has a low cost and can substitute or complement chemical fungicides (Jha & Anjaiah, 2007). In several parts of the world, both rhizobacteria and secondary bacterial metabolites have been isolated and characterized for their potential to inhibit the phytopathogenic fungi of agricultural crops (Jha & Anjaiah, 2007; Jung *et al.*, 2014; Seiber *et al.*, 2014). Antagonist bacteria are characterized for their capacity to produce antibiotics, siderophores, hydrogen cyanide, chitinases, glucanases, and proteases (Nagarajkumar *et al.*, 2004; Babalola, 2010). These characteristics give them the ability to inhibit phytopathogen growth (Jung *et al.*, 2014).

In chili crops, obtaining healthy seedlings in seedbed is essential to avoid the spread of diseases when they are transplanted into the field. This can be done with the use of phytopathogen-free substrates or by applying chemical or biological fungicides during seedling growth. One of these fungicides could be rhizobacteria with antifungal activity. The objective of this study was to characterize the ability of three rhizobacterial strains to inhibit the growth of *Rhizoctonia solani* and to test its potential in chili seedbeds as biocontrol agents.

Material and methods

Microbial strains

The three rhizobacterial strains used in this work were selected from the collection at the Laboratory of Microbiology of the Colegio de Postgraduados, Mexico. Strains A46 and P61 were isolated from the rhizosphere of potatoes, whereas strain S108 was isolated from the sporome of the ectomycorrhizal fungus *Suillus* sp. All three strains were grown in nutrient agar medium at 28°C. The strain of the phytopathogenic fungus *R. solani* was isolated from lettuce (*Lactuca sativa*) and selected based on a pathogenicity test in chili seedlings. Briefly, this was done as follows: Serrano chili seeds were placed on filter paper in magenta boxes GA-7 (77 mm × 77 mm × 97 mm) and sterile distilled water was added. After germination, two mycelial plugs taken from a five day grown culture were placed into the boxes and the number of dead plants was recorded for 10 days. The *R. solani* strain was preserved in potato dextrose agar (PDA) until it was used.

Molecular identification of the rhizobacerial strains

The genetic material was extracted from biomass equivalent to seven colonies of each strain from young cultures of 24 h with an EZ-10 Spin® Column Bacterial DNA mini-prep kit. The amplification of a ~1,500 bp fragment of the 16S rDNA was performed for reliable identification to the level of genus and species. The primers used for this amplification of the 16S rDNA were 27F (forward) 5′-AGA GTT TGA TCM TGG CTC AG-3′ and 1492R (reverse) 5′-CGG TTA CCT TGT TAC GAC TT-3′.

The PCR products were purified with an Agencourt® AMPure® XP kit, following the manufacturer's instructions. The purified amplicons were sequenced at MACROGEN, Korea.

The sequences were edited with BioEdit software 7.0.9.0 and Seaview 4.0. With the sequences, an analysis in the BLAST (http://www.ncbi.nlm.nih.gov/BLAST) of the National Centre of Biotechnology Information (NCBI) platform was performed to search for homologous genes for identification at the species level with identity percentages higher than 97%. The 16S rDNA gene sequences were deposited in the GenBank data base.

Antagonistic activity against *Rhizoctonia*

The rhizobacterial strains were streaked in lines on PDA medium. After 24 h, an agar disc with *R. solani* mycelium was placed 5 cm away from the bacterial culture, and the plates were incubated at 28°C. After 5 days, the radial growth of the phytopathogenic fungus was measured. The percent inhibition was calculated using the following formula: % Inhibition = (R–r)×100/R, where R is the radius of the fungus in the control and r is the radius of the fungus in the presence of the antagonist. The experiment was set up in a completely randomized design with three replicates per treatment, and it was repeated three times. The fungus radial growth data were analyzed in SAS 9.0, and the treatment means were compared using Tukey's test ($p \leq 0.05$).

Detection of in-plate siderophore production

The rhizobacterial strains P61, A46, and S108 were plated on chrome azurol S (CAS) agar, as described in brief as follows. The CAS dye was prepared according to Louden *et al.* (2011). The rhizobacterial strains were cultured in nutrient broth for three days at 28°C under shaking conditions. The cultures were then centrifuged at 7,000 rpm for 15 minutes. The supernatant was removed and the pellet was washed three times with double-distilled water to remove any trace of oligoelements. The pellet was resuspended in 5 mL of double-distilled water. Plating was performed by adding 10 µL of the bacterial suspension to the plates

containing CAS medium to obtain separate colonies. The formation of yellow to orange halos around the colonies was evaluated after 72 h of incubation at 28°C (Schwyn & Neilands, 1987; Milagres *et al.*, 1999; Shin *et al.*, 2001; Pérez *et al.*, 2007). The experiment was set up in a completely randomized design with five replicates per treatment. The data were analyzed in SAS 9.0 and the means of the halos of each strain were compared using Tukey's test ($p \leq 0.05$). This experiment was repeated three times. Additionally, an antagonism experiment using the rhizobacterial strains against the fungus in dual culture was performed in PDA medium with and without iron ($FeCl_3$, 0.1 g/L) to determine if the siderophore production of the strains is responsible for inhibiting the growth of the phytopathogenic fungus.

Detection of antifungal volatile compounds

The rhizobacterial strains (P61, A46, and S108) and the phytopathogenic fungus were plated on divided PDA plates. The bacteria were plated on one section, and after 24 h, a disc containing *R. solani* was placed in the other section of the plate. The fungal growth was measured after 5 days and was compared with that of the controls (Velázquez-Becerra *et al.*, 2011).

Detection of chitinolytic activity in plate

This test was carried out according to the method described by Inglis & Peberdy (1997) with a slight change. In brief, rhizobacterial strains P61, A46, and 108 were grown in Luria Bertani (LB) medium added with 1% colloidal chitin (Sigma), previously digested with concentrated hydrochloric acid for 24 h. The plates were incubated at 28°C for 72 h. The formation of a halo around the colonies was evaluated.

Bacterial filtrate assays

Two assays using filtrates from the bacterial strains were performed. In the first assay, the strains were cultured in potato dextrose broth (PDB) for 72 h under shaking at 28°C. The culture was centrifuged at 7,000 rpm for 15 min, and the supernatant was filtered using Millex 0.22-µm Durapore membranes. Each bacterial filtrate was incorporated into autoclaved PDA cooled to 50 °C to obtain 1:9 and 3:7 ratios (v/v, filtrate/PDA), and finally the media were poured in petri plates; a control with no filtrate was included. A *R. solani* disc was placed at the center of PDA+bacterial filtrate plates. The diameter of the fungal growth was measured after 48 h.

In the second experiment, the rhizobacteria were co-cultured with the fungus (*R. solani*) on PDB. First, an agar disc containing *R. solani* was inoculated, and 24 h afterwards, the bacterial strain was inoculated (one loop) and incubated at 28°C under shaking for 72 h. The microbial cultures were filtered with 0.22-µm Durapore membranes; the broths were added into PDA as described above to obtain 1:9 and 3:7 ratios (filtrate/PDA). Next, an *R. solani* disc was placed in the plates that contained medium. A control without filtrate was included. The cultures were incubated at 28°C. After 48 h, the development of *R. solani* was evaluated. In both assays, the experimental design was completely randomized, and it included three replicates per treatment. The data were analyzed in SAS 9.0, and the fungal diameter means were compared using Tukey's test ($p \leq 0.05$).

Additionally, a pH test was performed to observe the effect of pH following bacterial growth in each of the filtrates. This test was based on adjusting the initial pH of the medium to 7. The PDB medium was then sterilized, and each rhizobacterial strain was inoculated and incubated for 72 h under shaking at 28°C. The final pH of each bacterial strain grown in liquid medium was measured and re-adjusted to 7, followed by the filtration of the supernatant. In this experiment, the effect of the pH of the bacterial filtrates on *R. solani* growth was evaluated. The experimental design was completely randomized with two treatments (adjusted and non-adjusted pH) and six replicates.

Biocontrol test with rhizobacteria strains in plants

The bacterial strains were used to establish an assay in Serrano chili (*Capsicum annuum* L.) seedlings. The bacterial strains were cultured in PDB under shaking at 28°C for 72 h. The cultures were then centrifuged for 15 min at 7,000 rpm, and the pellets were re-suspended in distilled water to obtain a final concentration of 10^8 bacterial cells/mL. Trays with 200 cells were filled with a previously sterilized mixture of agrolite, peat moss, and vermiculite (1:1:1). Three seeds were planted in each cell, and after seedling emergence, the cells were thinned to only one seedling per cell. Then, 3 mL of bacterial suspension was added per seedling. Five treatments were included, the three strains, one with fungicide (Captan, 2 g/L), and the control with sterile distilled water only. Captan was applied only once using a volume of 3 mL per seedling. The phytopathogenic fungus *R. solani* was cultured in PDA plates for 5 days to obtain the inoculum; one disc containing mycelium was placed per seedling. The relative humidity around the seedlings was increased by covering them with plastic bags to accelerate the onset of the disease. Data about the disease symptoms were taken from the aerial parts of the seedlings, and the number of dead seedlings per treatment was counted periodically.

The experiment with chili seedlings in a greenhouse was established in a randomized block experimental design, in which the treatments were as follows: bacterial strains, Captan, and a control, each with four replicates of 40 seedlings. The cumulative number of dead seedlings per treatment determined at each date was considered separately for analysis of variance and then Tukey's test ($p \leq 0.05$) was performed using a multiple comparison of means in SAS 9.0.

Table 1. Molecular identification of strains according to 16S rDNA amplification sequences compared to GenBank.

Isolate	bp	GenBank accession number	Query coverage	Max. identity (%)
P61	1365	FM202487. *Pseudomonas tolaasii*	100	99
A46	1279	FM202487. *Pseudomonas tolaasii*	100	99
S108	800	AM268337.1 *Ranhella aquatilis*	100	99

Results

The sequences of the strains P61 and A46 had 99% similarity to the sequence of *Pseudomonas tolaasii*. In the case of strain S108, the sequence showed 99% similarity to the sequence of *Rahnella aquatilis* (Table 1). This strain also had similarity with the genera *Ewingella, Hafnia, Serratia*, and *Rouxiella*. According to its phenotypic characteristics, S108 fits better with the description of *Rhanella* (Kämpfer, 2005; Brady *et al.*, 2014). The strain S108 has creamy-colored, convex colonies with full margins, is negative for indole production, grows at temperatures between 4 and 37°C, and the cells are motile. The nucleotide sequences were submitted to the NCBI GenBank database, where the following accession numbers were assigned: KY933651.1 (strain A46), KY933652.1 (strain P61), and KY933653.1 (strain S108).

Antagonistic activity

The three rhizobacterial strains tested in this study in dual culture showed antagonistic activity against *R. solani*. Strains P61 and S108 inhibited the radial fungal growth by 49%, whereas strain A46 inhibited it by 37% (Table 2). The formation of the inhibition zone of the fungus was observed in the presence of the bacterial colony in each of the rhizobacterial treatments.

Detection of in-plate siderophore production

Strains A46, P61, and S108 were positive for the siderophore tests; strains A46 and P61 formed yellow halos around the colonies. Regarding strain S108, an orange halo was observed; strains P61 and S108 showed wider halos, indicating a greater production of siderophores (Table 2). The experiment using PDA supplemented with available iron did not reduce the inhibitory activity of the rhizobacteria on the fungus (data not shown). This finding suggests that the antagonism exerted by the strains on the fungus is not due to siderophore production.

Detection of antifungal volatile compounds and chitinolytic activity

No inhibition of *R. solani* radial growth was observed in the divided dishes with the fungus growing on one section and the bacterial strain on the other (Table 2). The fungus grew to surpass the plate division, and once it crossed the division, the formation of an inhibition zone caused by the presence of strains P61, A46, and S108 was observed. This finding suggests that no organic volatile antifungal compounds were produced. With respect to chitinolytic activity, there was no formation of halos around colonies. This indicates that the strains did not produce chitinases.

Antifungal activity of the rhizobacterial filtrates

The filtrate obtained from strain S108 and added to PDA at 3:7 ratio showed the highest antifungal activity (Figs. 1 and 2). This antagonistic activity is possibly due to the metabolites that are produced by strain S108. The

Table 2. Characterization of three rhizobacterial strains according to their antagonistic activities.

Strains	Species	Inhibition (%) of *R. solani*	Radial growth (mm) of *R. solani*	Siderophore production (halo width, mm)	Volatile antifungals
Control	--	--	70 a	--	--
A46	*P. tolaasii*	37	44 b	7.0 b	--
P61	*P. tolaasii*	49	36 c	13.8 a	--
S108	*R. aquatilis*	49	36 c	12.8 a	--

-- No effect. Means followed by the same letters are statistically equal (Tukey's, $p \leq 0.05$).

antifungal activity of the filtrates from strains P61 and A46 against *R. solani* was low at both concentrations used in the PDA (Fig. 2A). This finding indicates that when the concentration of the filtrate increased in the culture medium, the effect was higher, particularly in the filtrate from strain S108, which was highly significant compared to the filtrates of the other strains (Fig. 2A). A slight acidification of the PDB was detected after the bacterial strains grew. To verify that the pH change was not responsible for the antifungal activity of the bacterial filtrates, the pH was adjusted to 7. This test showed no significant changes in the antifungal activity of the filtrates after the pH was neutralized (data not shown). The filtrates from strains S108 and A46 that were obtained from co-cultures of the rhizobacteria with the fungus showed inhibitory activity against *R. solani*. However, the filtrate from P61 had no effect (Fig. 2B).

Biocontrol activity in plants

The application of the rhizobacterial strains to Serrano chilli decreased the *R. solani*-related mortality rate of the seedlings in comparison with the control, being the strain S108 the best one. The effect of this strain was statistically equal to the effect obtained with the Captan treatment (Fig. 3).

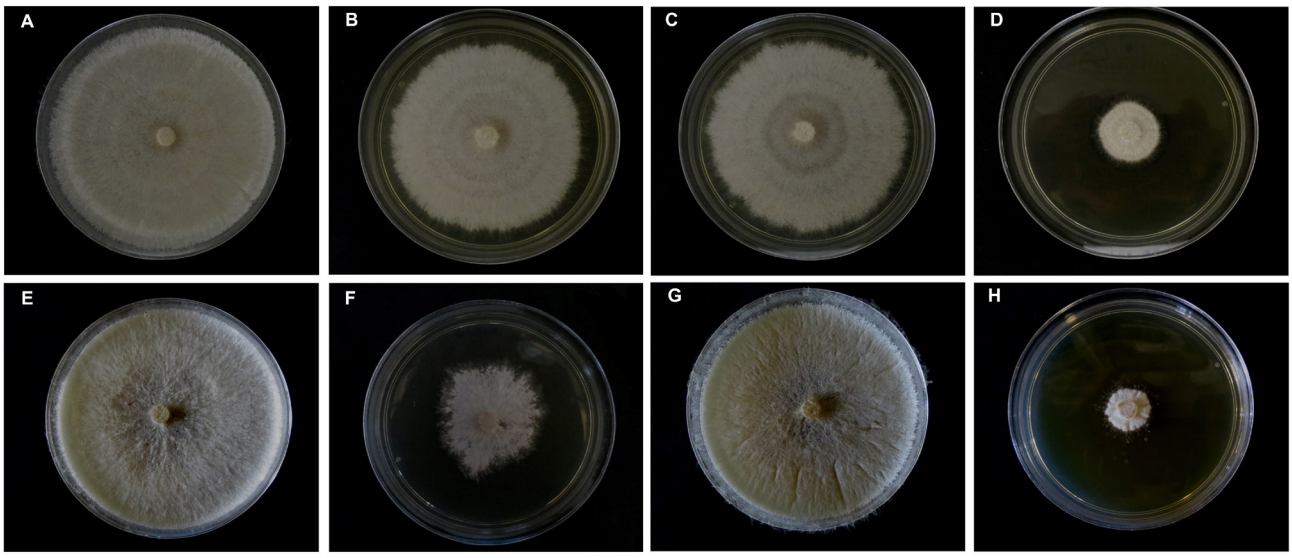

Figure 1. Growth inhibition of *R. solani* due to the addition of bacterial filtrates at 3:7 ratio in PDA medium. A to D: assay with filtrates obtained from bacterial cultures without the fungus. E to H: assay with filtrates obtained from bacterial co-cultures with the fungus. A and E, Controls. B, C, D: A46, P61 and S108 filtrates, respectively. F, G and H: A46, P61 and S108 filtrates from co-culture, respectively.

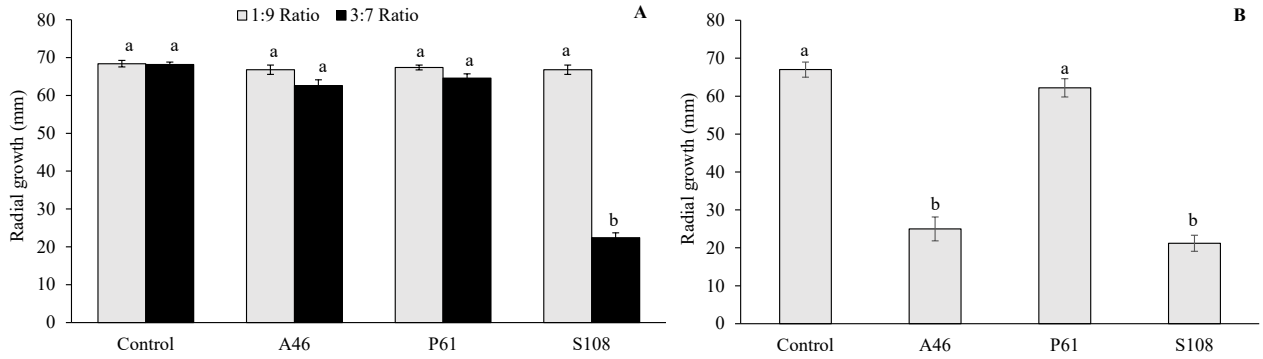

Figure 2. Inhibition of the radial mycelial growth of *R. solani* after applying filtrates from *Pseudomonas tolaasii* P61, *P. tolaasii* A46 and *Rahnella aquatilis* S108. A): Filtrates from bacterial cultures that were applied at two different ratios in PDA medium (1:9 and 3:7). B): Filtrates obtained from the co-culture of the rhizobacteria with the fungus that were added to PDA medium at 3:7 ratio. Identical letters over the bars indicate that the means were statistically similar (Tukey's test, $\alpha = 0.05$).

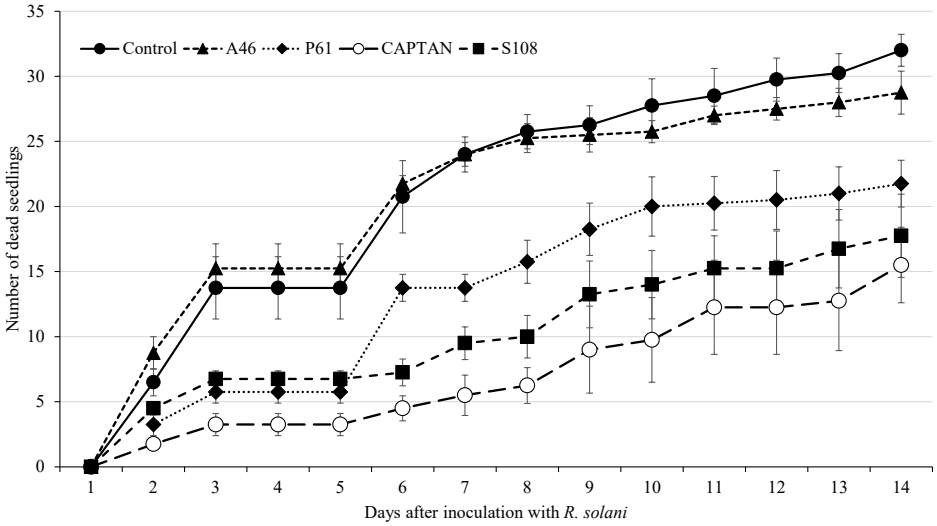

Figure 3. Effect of the application of three rhizobacterial strains on the mortality of Serrano chili seedlings that were inoculated with *Rhizoctonia solani*.

Discussion

All three rhizobacterial strains used in this study inhibited the growth of *Rhizoctonia solani* in dual culture (Table 2). These strains have the potential to control diseases in agriculturally important crops. Biological control using rhizobacteria is linked to their allelochemical activity, which includes the production of antibiotics, lytic enzymes, bioacids, molecules involved in quorum sensing, virulence factors, and siderophores (Chauhan *et al.*, 2014; Saraf *et al.*, 2014). In addition, it also involves competition for space or nutrients and the induction of systemic resistance in plants (Sulochana *et al.*, 2014).

In this study, several experiments were conducted to determine the ability of rhizobacterial strains to inhibit the growth of *R. solani*; their antagonistic activity is not caused by the release of volatile organic compounds. This conclusion was supported by the divided plate experiment (in which there was a barrier in the middle of the plate), in which the phytopathogenic fungus grew, crossing the barrier on the fourth day, and once it passed the division, the inhibition zone was observed (data not shown). Other studies have demonstrated that rhizobacteria can control phytopathogens via the production of volatile compounds, primarily hydrogen cyanide. For example, *Pseudomonas* and *Bacillus* inhibit fungal development via HCN production (Zhang *et al.*, 2015).

The production of siderophores is another biocontrol mechanism that rhizobacteria have to suppress the growth of fungi that cause diseases in crops (Chernin *et al.*, 1995; Kielak *et al.*, 2013; Brzezinska *et al.*, 2014). The three rhizobacteria considered in this study produced siderophores (Table 2). These compounds chelate iron, are quite diverse, and can only be synthesized by plants and microorganisms like bacteria and fungi (Milagres *et al.*, 1999; Shin *et al.*, 2001; Pérez *et al.*, 2007; Louden *et al.*, 2011). It has been observed that when rhizobacteria take up the iron in the medium, they make it unavailable for other microorganisms, including phytopathogens, suppressing their growth. In this case, the production of siderophores was not the mechanism that was involved in the antifungal activity of the strains against *R. solani* because even in the PDA supplemented with iron, these strains inhibited the growth of the phytopathogenic fungi in the same manner as they did in the medium without iron (data not shown). In other studies, the production of chitinases by rhizobacterial strains of *Pseudomonas, Serratia*, and *Bacillus* has been reported and related to the antifungal activity of these microorganisms (Brzezinska *et al.*, 2014; Akocak *et al.*, 2015). In this work, none of the three strains produced chitinases.

The bacterial filtrates at a 1:9 ratio (filtrate/PDA) did not have antifungal activity on *R. solani*, probably because the amount of antifungals was low. By increasing the bacterial filtrates to a 3:7 ratio, it was found that the S108 filtrate showed activity against the fungus, this was with the filtrate obtained from bacterial culture without the fungus or co-culture with the fungus, which suggests that strain S108 releases compounds with stronger antifungal activity. The same was true with the filtrate A46 obtained from co-culture (Fig. 2).

The production of compounds of microbial origin is influenced by several factors including temperature, culture medium (nutrients), pH, the growth phase of the

microbial colony, stress conditions, and the presence of or stimuli from other microorganisms (Berendsen *et al.*, 2012; Zha *et al.*, 2013). In particular, strain A46 only released metabolites with antifungal activity when it was co-cultured with *R. solani* in PDB (Figs. 1 and 2). Contrastingly, strain S108 did not require the presence of the fungus to produce compounds with strong antifungal activity. Strain P61 without the fungi or in co-culture produced metabolites with weak antifungal activity.

Pseudomonas strains reportedly produce different antimicrobials like phenazine, 2,4-diacetylphloroglucinol, pyrrolnitrin, hydrogen cyanide, and pyoverdine, among others (Coraiola *et al.*, 2006; Rokni *et al.*, 2012). In the particular case of *Pseudomonas tolaasii*, this species causes rot in edible fungi, and the extracted toxin, which is called tolaasin, has been associated with the disease caused by the bacterium (Cho & Kim, 2003; Sunhee *et al.*, 2011). In this study, the two *P. tolaasii* strains significantly inhibited *R. solani* growth in dual culture. Their antagonistic activity may be due to the production of tolaasin, even when the filtrates had very low antifungal activity. Other studies also report that bacterial filtrates obtained from strains with antagonistic activity against fungi in dual culture do not always show antifungal activity (Li *et al.*, 2014).

In the case of *Rahnella aquatilis*, it is a Gram negative enterobacteria that has been found in water bodies, forest soils, mosses, colonized roots, and sporomes of ectomycorrhizal fungi (Izumi *et al.*, 2006; Opelt *et al.*, 2007; Degelmann *et al.*, 2009; Kumari *et al.*, 2013). This bacterial species has been reported as a plant growth promoting rhizobacteria (Kandel *et al.*, 2017) and it has antagonistic activity against phytopathogenic fungi of the genera *Penicillum, Botrytis, Fusarium, Rhizoctonia, Pythium*, and *Gaeumannomyces* (Calvo *et al.*, 2007; Opelt *et al.*, 2007; Kandel *et al.*, 2017). Other attributes that have been found in strains of this species are: the ability to produce secondary metabolites (peptides, siderophores, amino acids, organic acids, phytohormones) and solubilize phosphates (Pintado *et al.*, 1999; Opelt *et al.*, 2007; Oulkadi *et al.*, 2014; Kandel *et al.*, 2017). In this work, the filtrates of strain *R. aquatilis* S108 mixed with the PDA medium at 3:7 ratio showed high antifungal activity.

Rhizosphere microorganisms establish different interactions and produce a large variety of compounds with bioactivity that depends on the microorganism, the environment and the interaction itself (Cray *et al.*, 2015), so the use of two or more microorganisms in co-culture could be essential for the production of bioactive compounds, simulating what happens in the rhizosphere (Bertrand *et al.*, 2014). The co-culture of two microorganisms has been shown to increase the production of metabolites that are not present in individual cultures (Pettit, 2009; Bertrand *et al.*, 2014; Dashti *et al.*, 2014). For this reason, an experiment in which the bacteria were co-cultured with the pathogenic fungus was conducted in PDB, and the resulting filtrates were evaluated for their antifungal activity. Only the antifungal activity of the filtrate from the *P. tolaasii* A46 strain increased significantly due to the effect of co-culturing with the fungus, suggesting that the production of antifungal metabolites increased due to the presence of *R. solani*. This finding demonstrates that some rhizobacterial strains with the potential for the biological control of phytopathogenic fungi require fungal stimulus to trigger the production of antifungal compounds. Several factors affect the growth of microorganisms in culture medium, and in turn, these microorganisms produce several compounds (Wang *et al.*, 2011; Zhao *et al.*, 2013; Dashti *et al.*, 2014; Djinni *et al.*, 2014). Some of these compounds are organic acids, such as lactic, phenylacetic and propionic acids, which lower the pH of the culture medium, inhibiting filamentous fungi (Lavermicocca *et al.*, 2003; Saithong *et al.*, 2010; Li *et al.*, 2014). In our study, we observed that the pH of the medium was slightly acidified with all three rhizobacteria. Thus, to rule out this possibility as the cause of the *R. solani* inhibition, the pH was adjusted to 7 after the filtrates were obtained. We found that even when adjusting the pH, the filtrates maintained their antifungal activity.

The application of the strains S108 and P61 to Serrano chili seedlings decreased the mortality rate caused by *R. solani*, compared with the control. Strain S108 and the Captan treatment had a similar effect on the reduction of seedling mortality caused by the phytophatogen, which was maintained until the end of the experiment. This suggests that strain S108 had an effectiveness equivalent to the chemical fungicide (Fig. 3). Strains S108 and P61 have great potential as biopesticides to control *R. solani* in chili seedbeds and possibly other transplant vegetables.

Obtaining healthy seedlings in seedbeds is essential for the successful production of chili crops in the field. The use of inoculants prepared with rhizobacteria like the *R. aquatilis* S108 strain can help small farmers to control *Rhizoctonia* and possibly other fungi that cause root diseases in the seedbeds, being of low cost and friendly to the environment. However, it is necessary that the study continues with field tests in order to evaluate if the inoculation of the strain S108 is as effective in the control of this disease as the use of chemical fungicides.

In summary, the three strains of rhizobacteria in dual culture inhibit the growth of *R. solani* by between 38 and 49%. Strains produce siderophores; however, the

antifungal activity was not related to these compounds. The filtrate of strain S108 had the greatest effect on *R. solani* with 56% inhibition when this is added to the PDA medium in a 3:7 ratio. The rhizobacterial inoculation reduced the mortality of seedlings caused by *R. solani*. The best treatments were the inoculation of strain S108 and the application of Captan, which had statistically the same effect. The strain of *Rahnella aquatilis* S108 has potential as a biopesticide to control *R. solani* in chili seedbeds and possibly the filtrate obtained from the bacterial culture also works to reduce the growth of the pathogen at the nursery level.

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