Revista Iberoamericana de las Ciencias Biológicas y Agropecuarias

ISSN: 2007 - 9990

https://doi.org/10.23913/ciba.v9i17.96

Artículos Científicos

Antagonismo de microorganismos nativos sobre Phytophthora infestans (Mont.) de Bary aislada de Solanum tuberosum L.

Antagonism of Native Microorganisms on Phytophthora Infestans (Mont.) de Bary Isolated from Solanum Tuberosum L.

Antagonismo de microrganismos nativos em Phytophthora infestans (Mont.) De Bary isolado de Solanum tuberosum L.

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Revista Iberoamericana de las Ciencias Biológicas y Agropecuarias

Ciba

ISSN: 2007 - 9990

Resumen

El manejo inadecuado de las enfermedades en cultivos de la papa origina que algunas especies patógenas generen resistencia a diversos plaguicidas. Esto incrementa el uso de agroquímicos que ocasionan contaminación en el suelo y el agua, lo cual daña la salud humana. *Phytophthora infestans* (Mont.) de Bary (causante del tizón tardío) limita la producción de papa (*Solanum tuberosum* L.) y provoca pérdidas económicas importantes. Este tubérculo se produce en la región nororiental de Puebla, México, y está documentada la presencia de tizón tardío, el cual es controlado con la aplicación de fungicidas sintéticos. En el presente estudio se evaluó la inhibición antagónica *in vitro* de dos microorganismos nativos (*Trichoderma* sp. y *Pseudomona* sp.), aislados de la rizósfera de una solanácea silvestre (en las inmediaciones de un cultivo de papa), sobre el desarrollo de *P. infestans* procedente de plantas infectadas. Se hicieron pruebas utilizando a los antagonistas de manera independiente y en combinación. Y se determinó el efecto del antagonista sobre el patógeno con base en el porcentaje de inhibición de crecimiento radial, en el caso del hongo el tipo de interacción hifal, la capacidad y forma antagónicas. Se observó que el hongo puede inhibir indirectamente el crecimiento de *P. infestans* (alrededor de 50 %), mientras que la inhibición directa con ambos microorganismos evaluados por separado es de más de 65 %. El tratamiento utilizando la combinación de *Pseudomona-Trichoderma* disminuyó el crecimiento en mayor proporción (80 %-92 %) que cualquiera de los antagonistas evaluados de manera independiente. A pesar de que la bacteria disminuye el crecimiento del hongo antagonista, *Trichoderma* sp. produce compuestos que afectan indirectamente al fitopatógeno, lo que origina un efecto sinérgico entre los dos microorganismos nativos para disminuir el desarrollo del patógeno en cultivos *in vitro*. Se considera importante diseñar una estrategia para la utilización de microorganismos presentes en los suelos de la región (nativos) como agentes que reduzcan la incidencia de tizón tardío.

Palabras clave: control biológico, *Pseudomona* sp., tizón tardío, *Trichoderma* sp.

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Abstract

The inadequate management of diseases in potato crops causes some pathogenic species to generate resistance to various pesticides, which increases the use of agrochemicals that cause pollution in soil and water, damaging human health. *Phytophthora infestans* (Mont.) de Bary (cause of late blight) limits the potatoes production (*Solanum tuberosum* L.) and causes significant economic losses. Potato is produced in the northeast region of Puebla, Mexico, and the presence of late blight is documented, which is controlled with the application of synthetic fungicides. In this study, the antagonist inhibition *in vitro* of two native microorganisms (*Trichoderma* sp. and *Pseudomonas* sp.) isolated from the rhizosphere of a wild *Solanaceae* (in the vicinity of a potato crop), was evaluated on *P. infestans* obtained from infected plants. Tests were performed using the antagonists independently and in combination. The effect of the antagonist on the pathogen was determined based on the percentage of radial growth inhibition, in the case of the fungus the type of hyphal interaction, the antagonistic capacity and form. The results show that the fungus can indirectly inhibit the growth of *P. infestans* (around 50%), while the direct inhibition, with both microorganisms evaluated separately, is more than 65%. The treatment using the combination of *Pseudomona-Trichoderma* decreased the growth in greater proportion (80%-92%) than any of the antagonists evaluated independently. Although the bacterium decreases the growth of the antagonist fungus, *Trichoderma* sp. produces compounds that indirectly affect the phytopathogen, causing a synergistic effect between the two native microorganisms to decrease the development of the pathogen in vitro cultures. We consider it is important to design a strategy to use native microorganisms present in the soils of the region as agents that reduce the incidence of late blight.

Keywords: biological control, *Pseudomona* sp., late blight, *Trichoderma* sp.

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Resumo

O manejo inadequado de doenças nas culturas de batata faz com que algumas espécies patogênicas gerem resistência a vários pesticidas. Isso aumenta o uso de agroquímicos que causam contaminação no solo e na água, o que prejudica a saúde humana. Phytophthora infestans de Bary (Mont.) (A causa da praga tardia) limita a produção de batata (Solanum tuberosum L.) e causa perdas econômicas significativas. Esse tubérculo é produzido na região nordeste de Puebla, México, e a presença de ferrugem tardia é documentada, a qual é controlada com a aplicação de fungicidas sintéticos. No presente estudo, avaliou-se a inibição antagonista in vitro de dois microrganismos nativos (Trichoderma sp. E Pseudomona sp.), Isolados da rizosfera de um solanum selvagem (nas proximidades de uma cultura de batata), no desenvolvimento de P. infestans de plantas infectadas. Os testes foram realizados usando os antagonistas independentemente e em combinação. E o efeito do antagonista no patógeno foi determinado com base na porcentagem de inibição do crescimento radial; no caso do fungo, o tipo de interação hifal, a capacidade e a forma antagônicas. Observou-se que o fungo pode inibir indiretamente o crescimento de P. infestans (cerca de 50%), enquanto a inibição direta com os dois microrganismos avaliados separadamente é superior a 65%. O tratamento usando a combinação Pseudomone-Trichoderma diminuiu o crescimento em uma proporção maior (80% -92%) do que qualquer um dos antagonistas avaliados independentemente. Embora a bactéria retarde o crescimento do fungo antagonista, Trichoderma sp. Produz compostos que afetam indiretamente o fitopatógeno, o que causa um efeito sinérgico entre os dois microrganismos nativos para diminuir o desenvolvimento do patógeno em culturas in vitro. Considera-se importante elaborar uma estratégia para o uso de microrganismos presentes nos solos da região (nativos) como agentes que reduzem a incidência de queima tardia.

Palavras-chave: controle biológico, Pseudomona sp., Ferrugem tardia, Trichoderma sp.

Fecha recepción: Julio 2019 **Fecha aceptación:** Diciembre 2019

Introduction

Potato crops (Solanum tuberosum L.) are attacked by phytopathogenic fungi, both in harvest and post-harvest, mainly in regions with humid climate (Pérez and Forbes, 2008). To reduce the damage caused by fungal diseases, agrochemicals (fungicides) are used, which in turn decrease the quality of food, contaminate the soil and water (Badii and Varela, 2015).

Potato cultivation occupies the fourth place among the most consumed foods in the world and its production is 320 million tons per year (Borba, 2008). However, its production is limited by the attack of Phytophthora infestans (hereinafter P. infestans), the cause of late blight. The traditional way to control this disease is to use chemical compounds that pollute and cause P. infestans to develop resistance, for example, metalaxyl (Romero, Lozoya, Mora, Fernández and Grünwald, 2012).

Due to the drawbacks in traditional production systems (using agrochemicals), it has been decided to look for alternatives to control fungal diseases through microorganisms that act as antagonistic of these pathogens (Aguado, Rascón and Luna, 2012; Altieri and Nicholls, 2008) .

Trichoderma is a genus with species listed as biological control agents, preventing phytopathogenic fungi from causing damage to the roots and aerial parts of the plant. It acts through direct mechanisms such as mycoparasitism or competition for space and nutrients; It can also develop indirect inhibition strategies such as antibiosis (synthesis of toxic metabolites or antibiotics of volatile or non-volatile nature), growth stimulation (solubilizes nutrients and activates phytohormones) and the induction of systemic resistance (González and Reséndiz, 2012).

The genus Pseudomona includes saprophytic bacteria that promote plant growth, with biopesticide, biofungicide and biofertilizer activity. Its mechanisms for the biological control of fungal diseases are similar to those of Trichoderma (Cano, 2011; Hernández, Heydrich, Velázquez y Hernández, 2006)

Martínez and Osorio (2007) they found that biosurfactants produced by Pseudomona fluorescens reduce up to 60% the incidence of P. infestans in potatoes, without inhibiting the development of other fungi and soil bacteria. In tomato crops (Lycopersicon esculentum Mill.) It was observed that different strains of Trichoderma inhamatum inhibit the in vitro growth of P. infestans and reduce the incidence of phytopathogen in the field (Puño et al., 2001).

Paucarima et al. (2014) evaluated the potential effect of microorganisms considering their origin, and found that antagonistic natives and growth promoters (Bacillus spp., Pseudomonas fluorescens and Burkholderia cepacia) inhibit the development of Rhizoctonia solani and P. infestans in potatoes. In another study, 10 different strains of Trichoderma were evaluated in the control of P. infestans and Sclerotinia sp .; Here they observed that the native strains have greater possibilities of adaptability and success compared to the strains currently available commercially (García et al., 2017)

The reviewed background suggests that Trichoderma sp. and Pseudomona sp. they are an alternative to reduce the damage caused by late blight, so these antagonistic microorganisms were isolated from wild Solanaceae, in the immediate vicinity of potato crop fields, to evaluate direct and indirect in vitro inhibition (using them separately or simultaneously) on P. infestans, also native to the region, isolated from a local potato crop infected with late blight in Zacapoaxtla, Puebla, Mexico.

Materials and methods

P. infestans It was isolated from tissue in potato crops with symptoms of late blight infection in the town of Xaltetela, Zacapoaxtla, in the state of Puebla, Mexico. The plant material was collected by directed sampling: parts were taken with symptoms of infection in leaves, stems and petioles; These were deposited in transparent plastic bags (14 x 20 cm). In the laboratory the samples were deposited in Petri dishes on wet filter paper and left 24 hours at room temperature. 2 cm2 fragments of infected tissue were immersed in 70% ethanol for 1 minute, and 3% hypochlorite for 30 seconds. The fragments were deposited in nutrient agar and incubated for 8 days at 25 ° C in the dark (Fernández et al., 2005). The identification and description of the morphological characteristics was made based on what was compiled by Erwin and Ribeiro (1996).

For the isolation of native antagonists, a wild nightshade was sampled near a potato crop in the town of Ixticpac, Zacapoaxtla, Puebla. The sample was collected (200 g of substrate) in the soil surrounding the roots at a depth of 5-10 cm. The substrate was transferred in plastic bags to the laboratory and stored at 4 ° C until processing.

To isolate Trichoderma sp. the modified protocol described by Kim et al. (2003). The soil (10 g) was homogenized in 90 ml of distilled and sterilized water (removing fragments of roots, wood and stones); It was placed under agitation for three to five minutes. Serial solutions 10 $^{-2}$ and 10-5 g / ml were made and seeded in papa-dextrose-agar medium (PDA); It was incubated at 28 ° C for 8 days. For the determination of the macroscopic characteristics plate cultures were made for 14 days, the color of the colony (front and back), shape and texture were evaluated. The identification and description of the microscopic characteristics was done with microcultures observing conidia (shape, pigmentation and ornamentation), conidiophores (central axis type, branch characteristics, pustule formation), phyloid (presence / absence intercalar fiálides grouping form) and clamidospores (unicellular / multicellular), based on a taxonomic key for Trichoderma and Gliocladium (Kubicek and Harman, 1998). A Zeiss brand optical microscope with integrated camera was used.

To isolate and identify strains of Pseudomonas, 10 g of soil (removing fragments of roots, wood and stones) were homogenized in 90 ml of sterile 0.1% water-peptone; stirred for three to five minutes. Serial solutions ($10^{-}2$-$10^{-}5$ g / ml) were made and of the last two solutions 100 µm were taken to sow them in duplicate on nutrient agar. The cultures were incubated at 28 ° C for 24 hours. For the identification of strains of Pseudomonas sp. the protocol proposed by Helguero (2010) and Burkholder (1957) was used.

In vitro antagonism of Trichoderma sp. versus P. infestans was checked by the dual culture method in PDA medium. In the Petri dishes, two culture segments were placed 70 mm away: at one end a 1 cm diameter disc of agar with mycelium of P. infestans and on the opposite end another disk of the same size with mycelium of Trichoderma sp . For the witness only one medium disk with P. infestans was added at one end. They were incubated at 28 ° C and the radial growth of the mycelium of the pathogen was measured every 24 hours. The percentage inhibition was calculated with the data.

In vitro antagonistic capacity (with a scale of one to five) of Trichoderma sp. on the pathogen according to the extent of the mycelium of the antagonist with reference to the control (Bell, Wells and Markaman, 1982) and the form of antagonism (physical, chemical, hyperparasitic or physical-chemical) according to the zone of contact between organisms and intensity in percentage (Davet, Artigues and Martin, 1981). To determine the type of hyphal interaction (curl, penetration, vacuolization or lysis) microcultures were used (Riddell, 1950).

The indirect effect of Trichoderma sp. in the inhibition of mycelial growth of P. infestans was performed with the protocol described by Dennis and Webster (1971). The process consisted of adding 8 ml of PDA medium in both caps of the 90 mm diameter Petri dishes. A 1 cm diameter agar disk containing the mycelium of P. infestans was placed on the upper lid, and another agar disk with mycelium of the Trichoderma sp. Strain was added to the lower lid, then the two were placed caps facing each other and sealed; for the control, only an agar disk without microorganisms (clean) was used instead of the antagonist. The plates were incubated at 28 ° C and the growth of the pathogen in the control box and in the treatment was measured every 24 hours. The percentage inhibition was calculated with the data.

In vitro antagonism of the strains of Pseudomonas sp. versus P. infestans was proven by growing in nutrient agar. The bacteria were sown by a striatum over the entire box with the strain of Pseudomonas sp. and in the center a 1 cm diameter disc of agar containing the mycelium of P. infestans was placed. For the control, only one agar disc with mycelium of P. infestans was added in the absence of the striatum of Pseudomonas sp. The cultures were then incubated at 28 ° C and every 24 hours the radial growth of P. infestans was measured and compared with the radial growth of the pathogen in the control boxes. The percentage of radial growth inhibition was calculated jointly.

To determine the in vitro inhibition of Trichoderma sp. in combination with Pseudomona sp. on P. infestans, Pseudomonas sp. in PDA making a striatum over the entire surface, at one end a 1 cm diameter disc of agar with mycelium Trichoderma sp. and at the other end another disc with mycelium of P. infestans was placed. For the control only one agar disc with mycelium of P. infestans was added in the absence of the antagonists. Subsequently the cultures were incubated at 28 ° C and the radial growth of P. infestans in the control and treatment was measured every 24 hours. The percentage inhibition of the pathogen was calculated with the data.

In all antagonism tests the percentage of radial growth inhibition (ICRC) was obtained according to Martínez and Solano (1994), using the formula PICR = (R1 - R2) / R1 x 100, where R1 is the radius of the pathogen in the control box and R2 is the radius of the pathogen in the antagonistic test at 24-hour intervals; the measurements stopped when the control box was completely covered by the fungus.

The tests were done with three repetitions per treatment and the final percentage inhibition value was compared to identify if there were significant differences between treatments. This by

means of an analysis of the variance (Anova) and a comparison of means by the Tukey-Kramer method at an α of 0.05, after having verified normality of data.

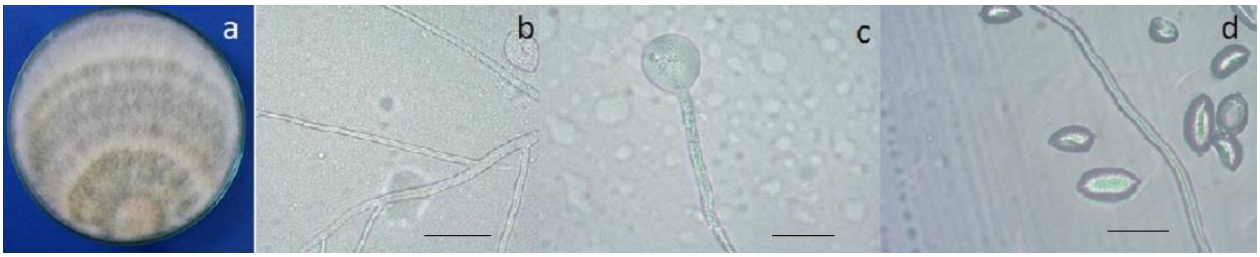
Results and Discussion

In the first phase the native antagonistic organisms and the phytopathogenic fungus were isolated and identified. Once the identity of the microorganisms was verified, the different inhibition tests were carried out.

Isolation and identification of microorganisms

The macroscopic and microscopic characteristics of P. infestans isolated from infected cultures are shown in Figure 1. The fungus grown in nutritive agar showed creamy pigmentation, with concentric zoning and cottony texture. In the microcultures distinctive characteristics of the species were observed: cenocitic hyphae, oogonium, sporangiophore and sporangia with the presence of pedicel and semipapilla.

Figura 1. *P. infestans* aislado a partir de tejido de plantas de papa infectados cultivado en agar nutritivo (a), y en microcultivos que permiten observar las hifas cenocíticas 10x (b), esporangióforos 100x (c), y esporangios con pedicelo y semipapila 100x (d). Las barras corresponden a 10 micrómetros en 100x y 100 micrómetros en 10x



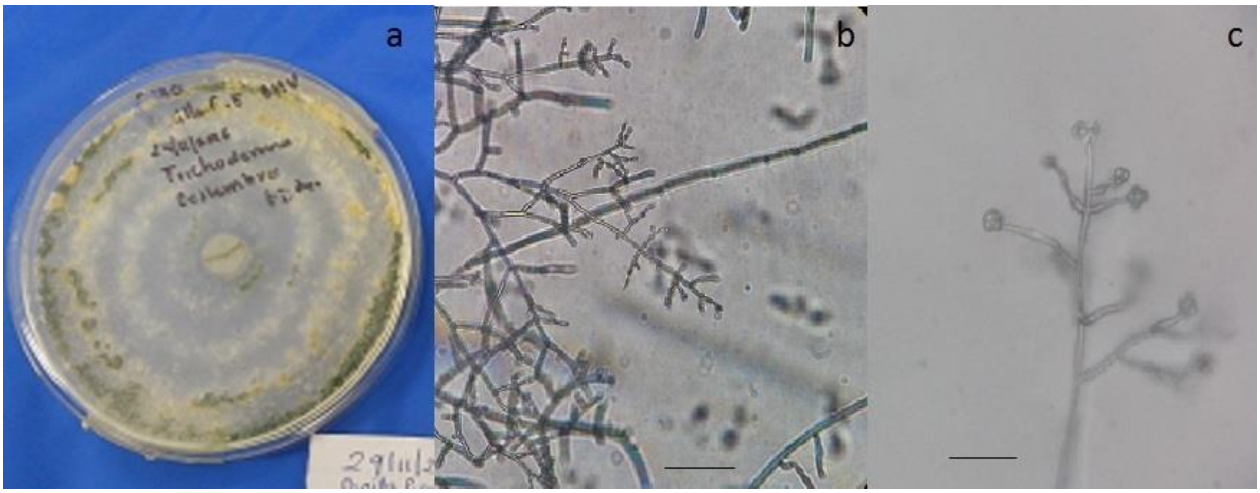
Fuente: Elaboración propia

It was observed that the macro and microscopic morphological characteristics coincide with the descriptions of Erwin and Ribeiro (1996).

The characteristics of the native strain of Trichoderma sp. isolated from soil in the rhizosphere of the wild Solanaceae of the locality of Ixticpac, Zacapoaxtla, is shown in Figure 2.

The colonies have a yellow-greenish color after 14 days of in vitro culture, form concentric rings of flocculent texture, with dense and colorless sporulation on the obverse of the inoculum.

Figura 2. *Trichoderma* sp. aislada a partir de suelo en la rizósfera de *Solanum* sp. silvestre de la localidad de Ixticpac, Zacapoaxtla, cultivado en PDA (a), los microcultivos que permiten observar los conidióforos y fiálides a 40x (b), y a 100x los conidios (c). Las barras corresponden a 10 micrómetros en 100x y 40 micrómetros en 40x



Fuente: Elaboración propia

The isolated strain has septal hyaline hyphae, in the central axes originated branched conidiophores with solitary phyloids in the form of elongated bottles, where the ovoid conidia are attached in the form of clusters. The arrangement of the fiálides is opposite or intercalated.

Cultures of bacteria isolated from the soil from the rhizosphere of the wild Solanaceae of the locality of Ixticpac, Zacapoaxtla, were identified as Pseudomona sp. according to the biochemical tests (table 1) and Gram staining.

Tabla 1. Pruebas bioquímicas a *Pseudomona sp*. aisladas a partir del suelo procedente de la rizósfera de una solanácea silvestre de la localidad de Ixticpac, Zacapoaxtla

Prueba	**Código**
Tinción de Gram	-
Crecimiento a 42 °C	-
Crecimiento a NaCl 6.5 %	-
Catalasa	+
Movilidad	+
Glucosa oxidasa	+
Lactosa-sacarosa-glucosa	+

Fuente: Elaboración propia

It should be noted that the differences in the location of where the antagonistic microorganisms and pathogens were obtained are due to the fact that in the potato crop fields in which the presence of late blight was detected, wild Solanaceae were not located, and vice versa: no presence of P. infestans was observed in the fields where wild nightshades surrounded the crop field.

Antagonism tests

In vitro antagonism of Trichoderma sp. versus P. infestans. Dual tests show that Trichoderma inhibits the growth of P. infestans more than 70% after 250 hours (Figure 3).

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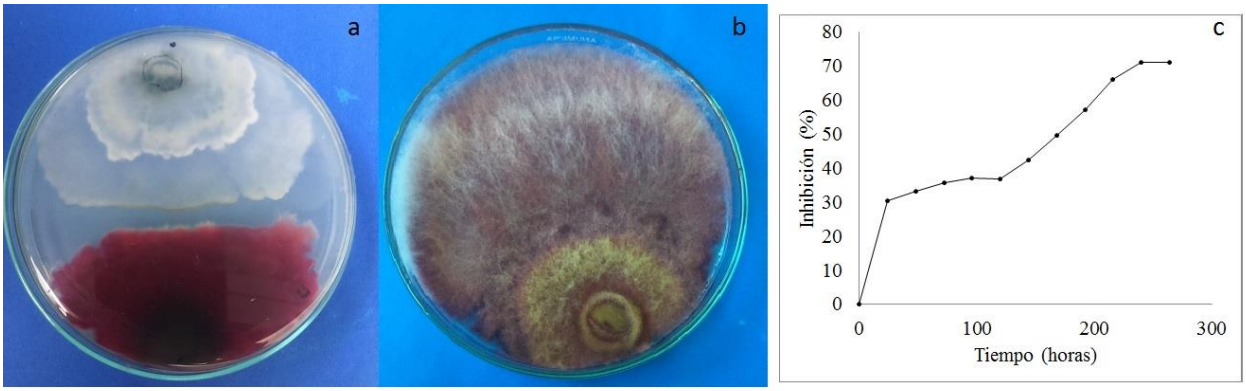
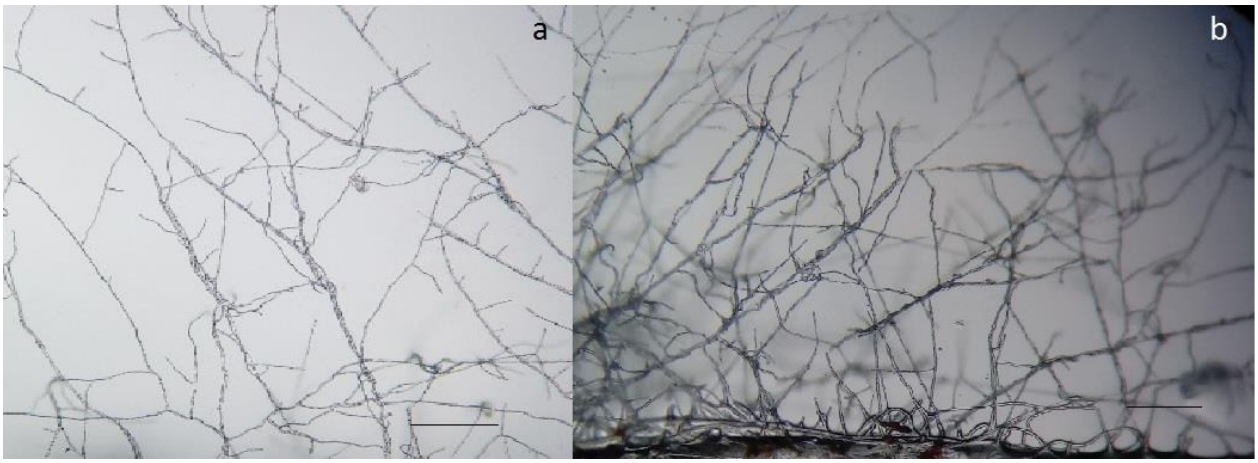
Figura 3. Inhibición *in vitro* de *Trichoderma sp*. sobre *P. infestans* vista del reverso (a) comparado con el testigo vista del anverso (b) cultivado en PDA, y monitoreando el porcentaje de inhibición durante 260 horas (c) a 28 °C en la oscuridad



Fuente: Elaboración propia

According to Bell et al. (1982, cited in Suárez and Cabrales, 2008), the antagonistic capacity of Trichoderma sp. It was grade three because, after 250 hours of incubation, the antagonist covered more than 50% of the surface of the box in the presence of the pathogen. A high antagonistic intensity was presented since P. infestans was inhibited more than 50%. The form of antagonism is hyperparasitic, since there is an overlap of mycelia, and the antagonistic fungus covers part of the parasite intertwining it. The hyphal interaction is by winding (figure 4).

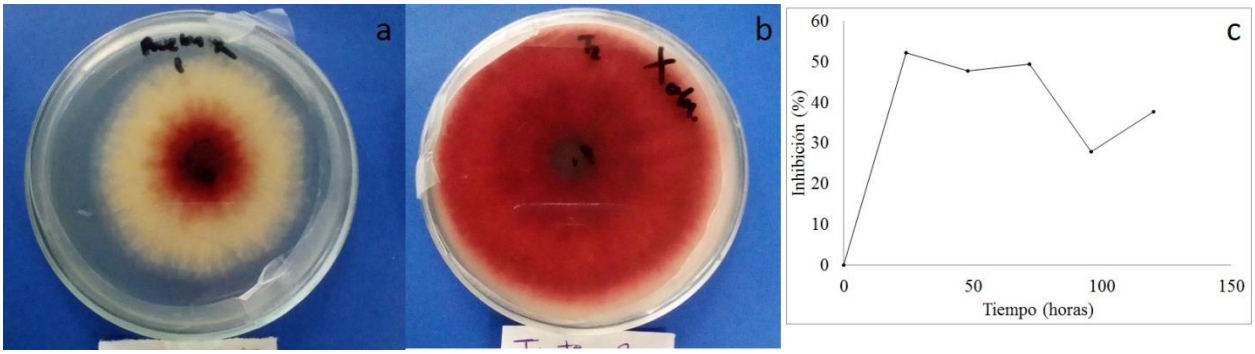
Figura 4. Microcultivo para evaluar el tipo de interacción antagonista entre *Trichoderma* sp. y *P. infestans* cultivado en PDA durante 96 horas a 28 °C en la oscuridad, observadas en el microscopio óptico a 10x (a) y 100x (b)



Fuente: Elaboración propia

In the test to evaluate the indirect effect caused by Trichoderma sp. on the growth of P. infestans it was observed that there is inhibition from the first monitoring at 24 hours, and the values range between 25% and 50% during the entire duration of the experiment; at 120 hours the phytopathogen is inhibited approximately 40% (figure 5).

Figura 5. Inhibición indirecta *in vitro* ocasionada por *Trichoderma* sp. sobre *P. infestans* comparado (a) con el testigo cultivados en PDA (b) y monitoreando del porcentaje de inhibición durante 120 horas (c) a 28 °C en la oscuridad. El microorganismo con coloración blanquecina corresponde al antagonista



Fuente: Elaboración propia

The antagonistic capacity is three, since from the first evaluation (at 24 hours) the inhibition is greater than 50%. Kumar et al. (2015) evaluated in vitro seven species of Trichoderma (isolated from different locations in Uttar Pradesh, in northern India), identified as wild by the authors themselves and obtained directly from the soil; They found that all are effective as antagonists of different phytopathogens, but the percentage of inhibition is different: Trichoderma reesei inhibits more than 60% of phytopathogenic species of the Alternaria Sclerotium and Bipolaris genera, while Trichoderma viride inhibits more than 80% of P. infestans . What was found in this work is close to the values mentioned above, since the antagonistic fungus inhibits the phytopathogen up to 70% at 260 hours of culture. The presence of Pseudomona sp. in the same culture system the growth of P. infestans decreases (figure 6).

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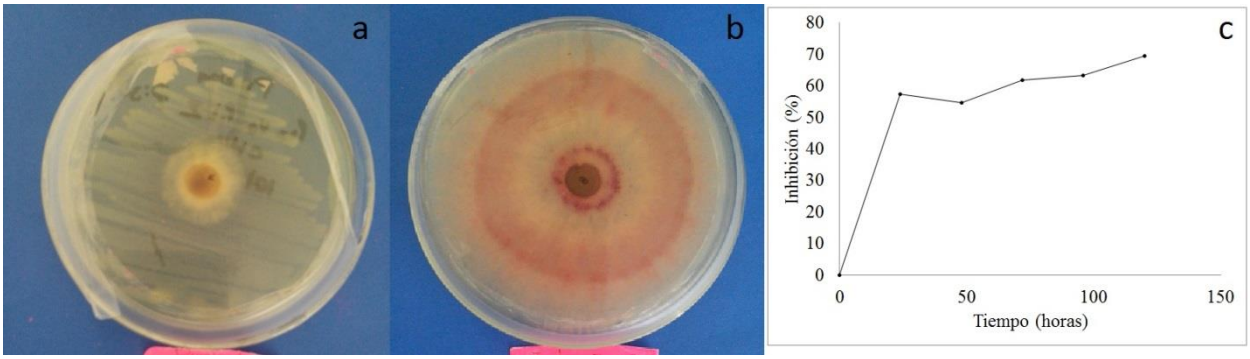
ISSN: 2007 - 9990

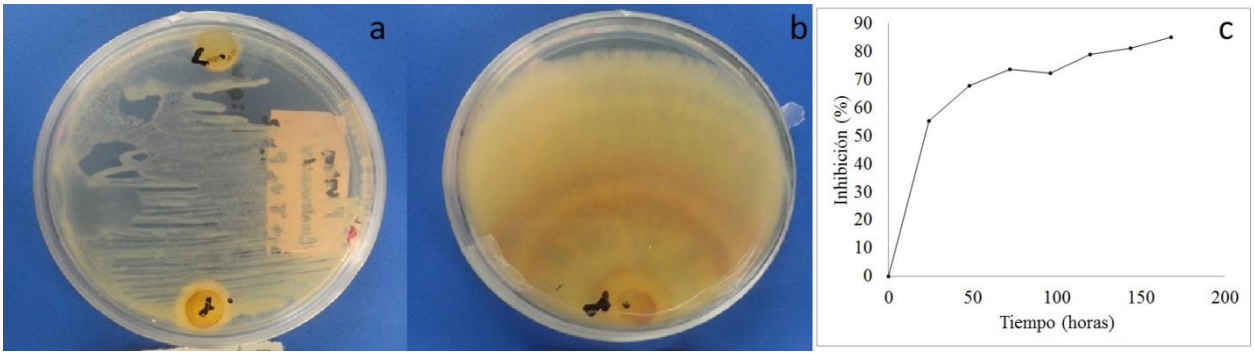
Figura 6. Inhibición *in vitro* de *Pseudomona* sp. sobre *P. infestans* (a) comparado con el testigo cultivado en PDA (b) y monitoreando el porcentaje de inhibición durante 120 horas (c) a 28 °C en la oscuridad. El estriado blanquecino es el antagonista



Fuente: original de los autores.

The results of the in vitro evaluation of the inhibitory effect of Pseudomona sp. and Trichoderma sp. on the growth of P. infestans for 168 hours. The presence of the bacterium inhibits the two seeded fungi, apparently Trichoderma did not present mycelial growth, and the presence of both antagonists causes P. infestans to be inhibited more than 80% after 120 hours, so the antagonistic capacity is considered level four.

Figura 7. Inhibición *in vitro* de *Pseudomona* sp. y *Trichoderma* sp. sobre *P. infestans* (a) comparado con el testigo (b) cultivado en PDA y monitoreando el porcentaje de inhibición durante 168 horas (c) a 28 °C en la oscuridad. El microorganismo estriado blanquecino es la bacteria



Fuente: Elaboración propia

The presence of Pseudomona sp. in combination with Trichoderma sp. it makes the inhibition of P. infestans more efficient than that observed when only one of the antagonists is present (table 2), considering that in 120 hours 80% is inhibited (value that was not obtained in the other tests); when native antagonists are found in the culture system independently, similar values are obtained (about 55%). However, the strains of Pseudomonas sp. not only did they counteract the growth of P. infestans, they also stopped that of Trichoderma sp. It is probably due to the low specificity of the compounds produced by the bacteria; for example, antifungal compounds produced by Pseudomona fluorescens have been detected that inhibit several species of *Phytophthora*, *Rhizoctonia* y *Fusarium* (Koche, Gade y Deshmukh, 2013).

Tabla 2. Porcentaje de inhibición al final de cada una de las pruebas en las que se evaluó el antagonismo de *Trichoderma* sp. (en contacto directo e interacción indirecta), *Pseudomona* sp. y la combinación *Trichoderma-Pseudomona* (silvestres) sobre *P. infestans*. Las letras representan diferencias significativa ($\alpha = 0.05$) entre promedios del porcentaje de inhibición con tres repeticiones, de acuerdo con un Anova y una comparación de medias por Tukey-Kramer

Tratamiento	**Inhibición (%)**	**Tiempo (horas)**
Trichoderma sp. en contacto directo	71b	264
Trichoderma sp. interacción indirecta (volátiles)	38ª	120
Pseudomona sp.	69b	120
Trichoderma sp.-*Pseudomona sp.*	85c	168

Fuente: original de los autores.

The difference in the percentage of inhibition of 85% at the end of monitoring on the phytopathogen when the two antagonistic microorganisms are present, in comparison with that obtained with the microorganisms individually (from 69% to 71%), suggests the production of compounds produced by the antagonistic fungus that contribute to the indirect decrease in the growth of P. infestans. Because the bacterium also inhibited Trichoderma and there was no mycelial contact as in the case of testing using only the antagonistic fungus. El-Hasan, Walker, Schöne and Buchenauer (2009) found volatile secondary metabolites produced by Trichoderma that inhibit the growth of phytopathogenic fungi such as Fusarium.

Regarding the in vitro inhibition of P. infestans by native microorganisms, it was determined individually that Trichoderma harzianum inhibits in vitro 86% the phytopathogen by

competition and colonization (Fatima, Noureddine, Henni and Mabrouk, 2015), while the The same inhibitory effect was observed by the combined inoculation of different strains belonging to the genus Pseudomona (De Vrieze et al., 2018). Likewise, Zegeye, Santhanam, Gorfu, Tessera and Kassa, 2011) found that species of the Trichoderma and Pseudomona genera are an alternative in the control of late blight in potatoes; However, this study indicates that microorganisms when inoculated individually are more effective than when they were added simultaneously, contrary to what was found in this study, since apparently there is a bacterial fungus synergism to inhibit the pathogen in conditions in vitro The above suggests that the presence of the soil of both antagonists is an alternative to reduce the impact of late blight on potato crops, inoculating these microorganisms to ensure the presence and effective density.

It is considered that tests should be carried out under regular cultivation conditions to design a strategy that allows the use of native microorganisms present in the soils of the region to reduce the incidence of harmful fungi such as that caused by late blight.

Conclusions

Native microorganisms belonging to the Pseudomona and Trichoderma genera isolated from the rhizosphere of a wild Solanaceae located in the immediate vicinity of potato crop fields (Solanum tuberosum) inhibit the in vitro growth of P. infestans from an infected late blight plantation.

The decrease in growth is presented by being in direct phytopathogen-antagonist contact, or indirectly in the case of the antagonistic fungus.

The results suggest that there is a fungal-bacterial synergistic effect, since the inhibition is percentage higher when both organisms are present simultaneously compared to individual inoculation; in all cases it was greater than 60% except in the case of indirect inhibition caused by *Trichoderma* sp.

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