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In vitro evaluation mycoparasitic capacity of *Irpex lacteus* P7B against fungi and oomycetes associated with plant diseases

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ABSTRACT

Background/Objective. Diseases of agricultural crops affect the yields and quality of products. Synthetic chemical compounds are generally used to control them; these cause harmful impacts to the environment, as well as to human health. In this sense, beneficial microorganisms can be used in agriculture as biocontrol agents, and contribute to obtaining food in sufficient and safe quantities. The fungus *Irpex lacteus* has been reported as a potential biocontrol agent. The objective of this research work was to evaluate the *in vitro* mycoparasitic capacity of the endophytic fungus *I. lacteus* P7B against 22 fungi and one oomycete associated with plant diseases.

Materials and Methods. The P7B isolate, previously detected as a mycoparasite, was used and molecularly identified by amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA, using primers ITS1/ITS4.The confrontations of the mycoparasite (P7B) against the phytopathogenic microorganisms were carried out in PDA culture medium. Three replicates were used for each microorganism, in addition to the controls, which consisted of placing the microorganisms individually.

Results. Molecular analyses determined that isolate P7B corresponded to *Irpex lacteus* (GenBank: PP922180). The results of the *in vitro* assays indicated that *I. lacteus* P7B inhibited all the phytopathogenic agents with which it was confronted, 100% inhibition by *I. lacteus* occurred approximately in 14 days, except for *Rhizopus* spp., this was at 23 days after the confrontations.

Conclusion. The present study demonstrates that the fungus *I. lacteus* presented 100% *in vitro* mycoparasitic capacity against the various fungi and an oomycete evaluated, so future work could focus on evaluating its mycoparasitic activity under field conditions.

Key words: biocontrol agent, phytopathogens, mycoparasite.

Introduction

Diseases caused by fungi and oomycetes currently cause important economic losses in crops (Meng *et al.*, 2009). On the other hand, the world population increase demands greater amounts and quality of agricultural products, and consequently, a greater use of pesticides to fight diseases (Lahlali *et al*., 2022). However, in recent years, consumers have become more aware of the side effects of the use of pesticides such as chemical fungicides on human health and the environment (Hou and Wu, 2010). One of the alternatives to reduce dependence on pesticides is biological control (Compant *et al*., 2005; Barratt *et al*., 2018), which is defined, in general terms, as any living microorganism (including viruses) used to fight a pathogen or pest by parasitism, antibiosis, competition for space or resources (Eilenberg *et al*., 2001; Stenberg *et al*., 2021). In this regard, several species of basidiomycete mycoparasites have been reported as potential biocontrol agents (White and Traquair, 2006; Pineda-Suazo *et al*., 2021). Mycoparasitism is a lifestyle in which the fungus establishes parasitic interactions with other fungi (Karlsson *et al*., 2017). Mycoparasitic fungi are enzyme producers with the ability to degrade the cell walls of fungi, allowing them to penetrate into other fungi to extract nutrients for their development (Cao *et al*., 2009). In this sense, the fungus *Irpex lacteus* is characterized by its saprophytic habit, although it has been proven to have a mycoparasitic behavior under certain conditions, implying interactions in which *I. lacteus* colonizes and obtains nutrients from other fungi by secreting diverse hydrolytic enzymes (Metreveli *et al*., 2014; Mezule a d Civzele, 2020; Gafforov *et al.,* 2023). The mycoparasitic abilities *I. lacteus* suggest possible applications in the biological control of phytopathogens in the agricultural context (White and Traquair, 2006; Sivanandhan *et al*., 2017; Yin *et al*., 2021). Due to this, the aim of this investigation was to evaluate the fungus *I. lacteus* (isolate P7B) *in vitro* with a dual confrontation against 22 fungi and one oomycete.

This work was conducted in the Plant Physiology and Biotechnology Laboratory of the Facultad de Ciencias Agropecuarias y Ambientales of the Universidad Autónoma de Guerrero (FCAA-UAGro), located in Iguala de la Independencia, Guerrero, México.

For this study, isolate P7B was taken from an endophytic mycoparasitic fungus, which was isolated from the asymptomatic area of the *Cedrus* sp. rhizosphere, identified molecularly by DNA extraction, and for this purpose, the internal transcribed spacer (ITS) region of the ribosomal DNA as amplified by PCR using the ITS1/ITS4 primers (White *et al*., 1990). DNA extraction, PCR, and sequencing were performed by the sequencing service of the Macrogen company (Macrogen, Inc., Seoul, Korea). The sequences obtained were edited and aligned using the MEGA X® program, and a consensus sequence was obtained, which was compared with those available in the GenBank.

Isolate *I. lacteus* P7B underwent a dual confrontation against 22 fungi and one oomycete associated to diverse diseases (Table 1), belonging to the collection of phytopathogenic fungi of the Plant Physiology and Biotechnology Laboratory of the FCAA-UAGro. Strains of phytopathogenic fungi and the mycoparasitic agent (P7B) aged 12 days, developed in a PDA medium. For the confrontation, a disk, 0.5 cm in diameter and with mycelia, was placed 1.0 cm from the edge of the Petri dish and each isolation was placed on the opposite side of the dish, equidistantly. Three repetitions were used for each fungus or oomycete, along with control treatments, which consisted in placing a mycelium disk from each microorganism on one side of the Petri dish. The culture media were placed at a temperature of 28 °C, and the area of inhibition was recorded using a millimeter ruler when the control treatments covered the entire surface of the Petri dish with a PDA medium, which occurred approximately 14 days after cultured. Exceptionally, the treatments confronted with *Rhizopus* spp. were incubated for approximately 23 days, since a slow mycoparasitism was observed for *I. lacteus* P7B for this genus. Photographs were taken of the advancement of the dual confrontation every 24 hours (Sony camera, Vario-Tessar®). Additionally, the area of interaction between microorganisms was analyzed against the antagonistic fungus *I. lacteus* P7B, in order to observe possible damages in the structures of the parasitized microorganisms, using a compound microscope (LABOMED®).

Based on the values registered of the confrontations between microorganisms and the fungus *I. lacteus* P7B, the percentage of inhibition was estimated using the formula = (D1-D2)/D1*100.

where:
D1= Mycelial diameter of the control
D2= Mycelial diameter of the confronted microorganism

The analysis of the consensus sequence in the GenBank with the BLAST tool showed that isolate P7B had a percentage of identity of 99.85% with *Irpex lacteus* (accession number JX290579). The consensus sequence derived from this study was deposited in the GenBank with accession number PP922180.

Table 1. Microorganisms used in the evaluation for the confrontation with *Irpex lacteus* P7Bfrom the collection of pyhtopathogenic fungi of the Plant Physiology and Biotechnology Laboratory, FCAA-UAGro.

Microorganisms	Identification method	Reference	Host	Key	Disease
Corynespora cassiicola	Morphological and molecular	Ortega-Acosta *et al*., 2020	*Hibiscus sabdariffa*	CC47GRO	Spotting of calyces
Colletotrichum gloeosporioides	Morphological and molecular	Cruz-Lagunas *et al*., 2020	*Citrus paradisi*	COLTOR1	Anthracnose on fruits
Rhizopus oryzae	Morphological and molecular	Palemón-Alberto *et al*., 2019	*Annona muricata*	ANTRAGUB	Soft rot of fruits
Phytophthora sp.	Morphological	NA*	*Carica papaya*	PAP-4	Associated with basal rot
Macrophomina sp.	Morphological	NA	*Arachis hypogaea*	C4	Associated with charcoal rot of fruits
Colletotrichum sp.	Morphological	NA	*Plumeria rubra*	CACALO1	Associated with anthracnose on leaves
Rhizopus sp.	Morphological	NA	*Stenocereus* sp.	RIPITA	Associated with soft rot of fruits
Phoma sp.	Morphological	NA	*Parmentiera* sp.	PHOCUA1	Associated with brown rot of fruits
Colletotrichum sp.	Morphological	NA	*Citrus paradisi*	TOCOCE	Associated with fruit anthracnose
Colletotrichum sp.	Morphological	NA	*Stenocereus* sp.	SALP	Associated with fruit anthracnose
Fusarium sp.	Morphological	NA	*Musa* sp.	FUPLA	Associated with fruit spotting
Alternaria sp.	Morphological	NA	*Passiflora edulis*	AL1	Associated with fruit spotting
Colletotrichum sp.	Morphological	NA	*Citrus paradisi*	TORTEPE	Associated with fruit rot
Colletotrichum sp.	Morphological	NA	*Eriobotrya japonica*	COLNISP	Associated with fruit anthracnose
Diplodia sp.	Morphological	NA	*Punica granatum*	GRANA1	Associated with fruit spot
Fusarium sp.	Morphological	NA	*Persea americana*	AGUAT	Associated with stem base rot
Colletotrichum sp.	Morphological	NA	*Mangifera indica*	COLMAN	Associated with fruit anthracnose
Colletotrichum sp.	Morphological	NA	*Mangifera indica*	COLCRIO	Associated with fruit anthracnose
Rhizopus sp.	Morphological	NA	*Carica papaya*	RIZOPAP	Associated with soft rot of fruits
Verticillium sp.	Morphological	NA	*Mangifera indica*	VERT1	Associated with vascular wilt of stem
Phoma sp.	Morphological	NA	*Crescentia alata*	CRES1	Associated with fruit rot
Phoma sp.	Morphological	NA	*Punica granatum*	GRANPIC	Associated with fruit spots
Penicillium sp.	Morphological	NA	*Allium sativum*	AJOPENI	Associated with bulb rot

*NA=Not applicable, identified morphologically.

In the *in vitro* evaluation of 22 fungi and one oomycete against *I. lacteus* P7B (Table 1), approximately 14 days later, it displayed 100% mycoparasitism on all the microorganisms it was confronted with (Figure 1), except for the genus *Rhizopus* (23 days). Figure 2 shows some representative examples of the confrontation between *I. lacteus* P7B against fungi and one oomycete, in which a clear gradual mycelial invasion was observed. By the end of the experiment, it was determined that the fungus *I. lacteus* P7B induced an inhibition of 100% in all confrontations (Figure 1, 2).

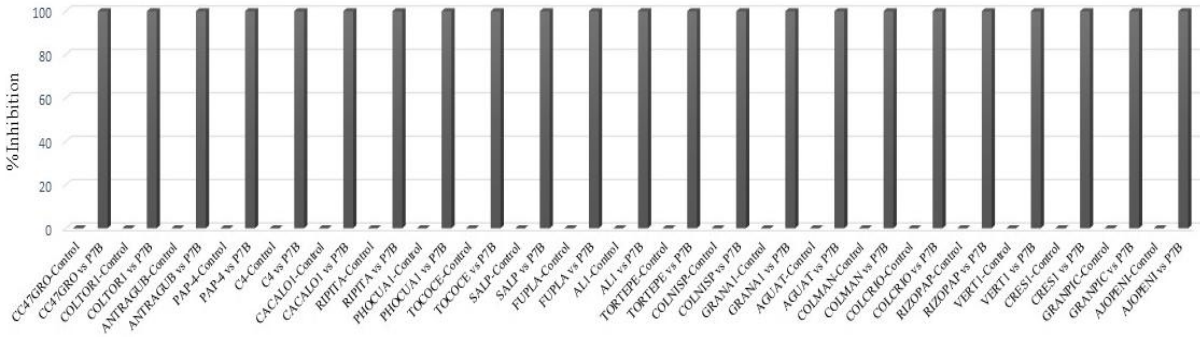

Figure 1. Effect of the confrontation in dual culture in PDA under *in vitro* conditions between *I. lacteus* P7B against fungi and an oomycete associated to plant diseases.

On the other hand, the fungi and an oomycete confronted with *I. lacteus* P7B all presented degradation of their structures when observed under the microscope. For example, *Macrophomina* sp. (isolate C4), in the zone of interaction, displayed degradation of sclerotia and hyphae (Figure 3B); *Alternaria* sp. (isolate AL1), it presented degraded conidia and hyphae (Figure 3D); for *Rhizopus* sp. (isolate RIZOPAP), degradation of sporangia was observed (Figure 3F); in control treatments, structures displayed no apparent damage (Figure 3A, C and E).

This work showed the mycoparasitic ability of *I. lacteus* P7B against 22 fungi and one oomycete associated to diverse phytosanitary problems. Literature on the potential of *I. lacteus* as a biocontrol agent is scarce. The fungus *I. lacteus* has the ability to produce diverse hydrolytic enzymes such as chitinases and glucanases, which degrade the cell walls of other fungi, facilitating the acquisition of nutrients (Qin *et al*., 2018; Roncero and Vázquez de Aldana, 2019). In a study carried out by White and Traquair (2006), by confronting *I. lacteus* against *Botrytis cinerea in vitro*, they proved that *I. lacteus* was able to parasite *B. cinerea* by degrading its structures such as conidiophores and conidia and parasiting its sclerotia, and reported a percentage of mycoparasitism of 100%, similar results reported in this

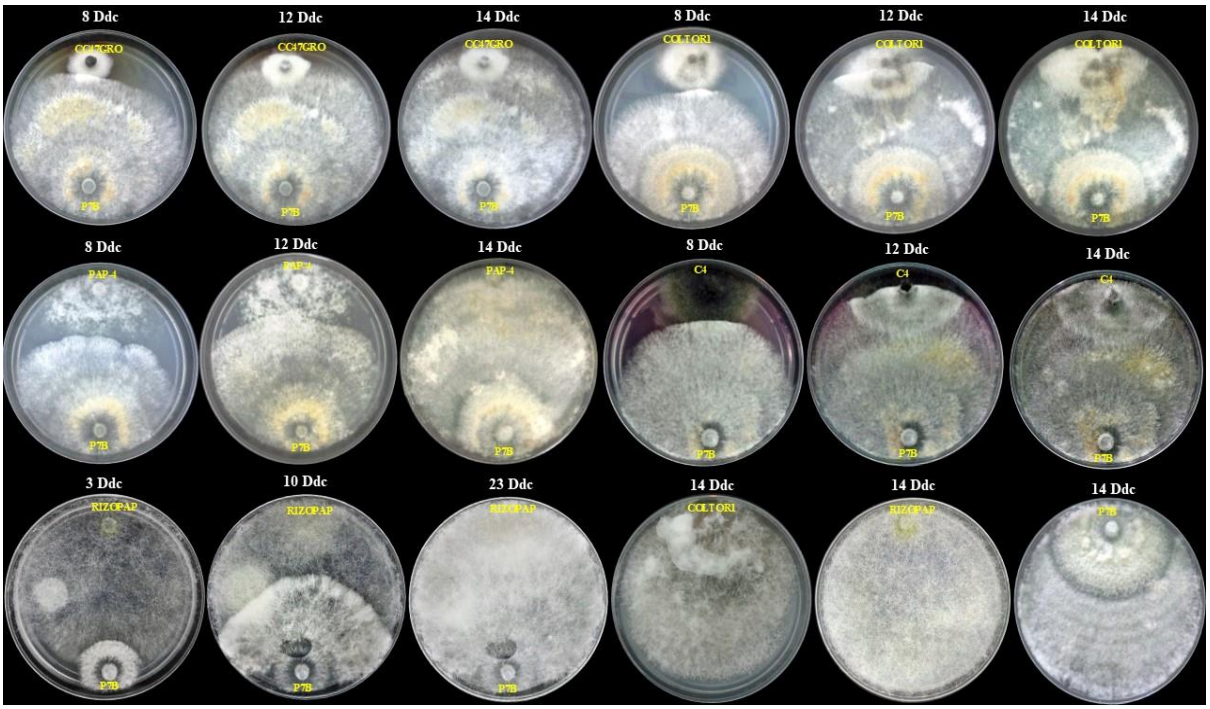

Figure 2. Effect of the confrontation in dual culture in PDA under *in vitro* conditions between *I. lacteus* P7B against fungi and an oomycete associated to plant diseases. P7B = *Irpex lacteus.* CC47GRO = *Corynespora cassiicola.* COLTOR1 = *Colletotrichum gloeosporioides.* PAP-4= *Phytophthora* sp. C4 = *Macrophomina* sp. RIZOPAP= *Rhizopus* sp. Dac = Days after confrontation.

study. On the other hand, in Mexico, *I. lacteus* has been evaluated against *Fusarium pseudocircinatum*, *F. mexicanum*, *Colletotrichum coccodes*, *C. gloeosporioides*, *Phytophthora capsici* and *P. cinnamomi* with a percentage of inhibition between 16.7 and 46.3% (Pineda-Suazo *et al*., 2021). In this investigation, *I. lacteus* P7B displayed a greater capacity for mycoparasiting diverse fungi and an oomycete, possibly due to the type of isolation. In addition, *I. lacteus* has been reported to belong to the group of necrotrophic mycoparasites, which are characterized for being highly destructive, scarcely specialized (Viterbo *et al*., 2007) and generally presenting a high range of hosts, including phytopathogens and extend to diverse taxonomic groups (Viterbo and Horwitz *et al*., 2010), as in this study, where *I. lacteus* parasite fungi and an oomycete of the divisions Ascomycota, Zygomycota and Oomycota. Additionally, compounds, derived from *I. lacteus* such as terpenes and aldehydes, have been detected which have an antifungal potential (Pineda-Suazo *et al*., 2021; Wang *et al*., 2021).

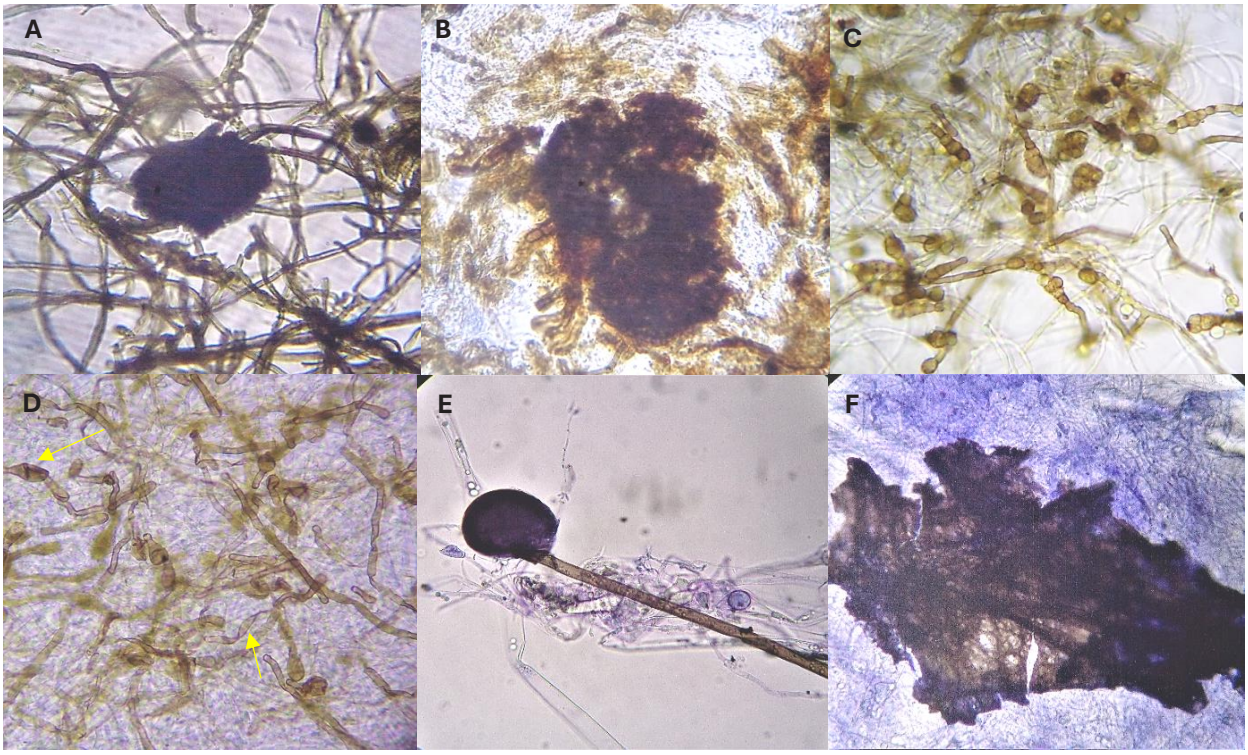

Figure 3. Effect of the confrontation *in vitro* of *I. lacteus* P7B against fungi and an oomycete. A= *Macrophomina* sp. (isolate C4) control; B= *Macrophomina* sp. (isolate C4) confronted with *I. lacteus* P7B, a degradation of sclerotia and hyphae can be observed. C = *Alternaria* sp. (isolate AL1) control; D = *Alternaria* sp. (isolate AL1) confronted with *I. lacteus* P7B, in which a degradation of conidia and hyphae can be observed. E = *Rhizopus* sp. (isolate RIZOPAP) control; F = *Rhizopus* sp. (isolate RIZOPAP) confronted with *I. lacteus* P7B, shows degraded sporangia. Images captured with an optic microscope with 10X (A, B, E and F), and 40X objective lens (C and D).

The fungus *I. lacteus* mycoparasited 100% *in vitro* 22 fungi and one oomycete evaluated in this study. Future investigations may focus on evaluating the antagonistic activity of *I. lacteus* under field conditions for the control of phytopathogens, as well as on the evaluation and determination of antifungal compounds derived from *I. lacteus* P7B.

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