Capacidades antagónicas de cepas *Trichoderma* y su multiplicación en masa usando desechos agrícolas

Antagonistic capacities of *Trichoderma* species and their mass multiplication with agricultural wastes

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Resumen

El objetivo de esta investigación fue aislar y caracterizar cepas de *Trichoderma* nativas de Misiones (Argentina) explorando sus capacidades antagónicas y su multiplicación masiva utilizando diferentes residuos agroindustriales. Quince cepas nativas de *Trichoderma* spp. fueron aisladas de muestras de suelo. Estos aislamientos se caracterizaron mediante observaciones morfológicas y moleculares basados en secuencias de ADN de la región espaciadora transcrita interna del ADNr. Las cepas de *Trichoderma* spp. fueron identificadas como *T. koningiopsis, T. harzianum, T. pleuroticola* y *T. brevicompactum*. Estas cepas mostraron actividades antagónicas in vitro contra *Alternaria* sp., *Fusarium* sp. y *Botrytis* sp.. *T. koningiopsis* LBM 090, LBM 091, LBM 092 y LBM 098, *T. pleuroticola* LBM 097 y *T. harzianum* LBM 096 presentaron una inhibición del crecimiento micelial mayor del 50% y un índice de antagonismo entre 3 y 4 contra los fitopatógenos ensayados. La cáscara de arroz y el pulido del arroz fueron las combinaciones más adecuadas para la multiplicación de *T. harzianum* LBM 096.

Palabras clave: Agentes de control biológico; Suelo; Hongos fitopatógenos; Cáscara de arroz; Pulido de arroz.

Abstract

The aim of this research was to isolate and characterize *Trichoderma* native strains from Misiones (Argentina) exploring their antagonistic capacities to phytopatogens fungi and their mass multiplication using different agricultural wastes. Fifteen native strains of *Trichoderma* spp. were isolated from soil samples. These isolates were characterized via morphological observations and molecular phylogenetic analysis based on DNA sequences of the rDNA internal transcribed spacer region. The *Trichoderma* native strains were identified as *T. koningiopsis, T. harzianum, T. pleuroticola* and *T. brevicompactum*. All strains showed antagonistic activities in vitro against *Alternaria* sp., *Fusarium* sp. and *Botrytis* sp. *T. koningiopsis* LBM 090, LBM 091, LBM 092, and LBM 098 strains, *T. pleuroticola* LBM 097 and *T. harzianum* LBM 096 presented radial mycelial growth inhibition higher than 50% and antagonism index between 3 and 4 against the phytopathogens assayed. Among the different substrate sources evaluated, rice husk and rice polishing were the most suitable combination for mass multiplication of *T. harzianum* LBM 096.

Keywords: Biological control agent; Soil; Phytopatogens fungi; Rice husk; Rice polishing.

INTRODUCTION

The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents (BCAs), which antagonistic properties are based on the activation of multiple mechanisms [1, 2, 3]. Antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications were carried out with different strains of the genus *Trichoderma* [3]. These properties made *Trichoderma* a ubiquitous genus present in any habitat and with high population densities.

Trichoderma strains are always, or frequently associated with plant roots and root ecosystems [4]. Some authors have defined *Trichoderma* strains as plant symbiotic opportunistic avirulent organisms, able to colonize plant roots by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defense mechanisms [5].

It is suitable to know the *Trichoderma* genus when it is introduced as a biocontrol agent into the rhizosphere of a given ecosystem for both control efficiency and environmental conservation reasons [6]. Therefore, it should be

considered morphological and molecular data from DNA sequencing to identify and characterize *Trichoderma* spp. [7]. One of the most reliable analyses for identifying a strain at the species level is the internal transcribed spacer (ITS) region [8].

Some of the phytopathogens that affect regional horticulture and plantation crops of Misiones province species are *Fusarium* sp., *Alternaria* sp. and *Botritis* sp. *Fusarium* sp. is reported as a soil pathogen that affects yerba mate nurseries [9]. *Alternaria* sp. and *Botritis* sp. are responsible for diseases in ornamental flowers and citrus plants [10, 11].

One of the widely used approaches to improve the agriculture production is the application of chemical pesticides. However, the degradation in the environment of such compounds is very difficult and the concentration and/or accumulation of them in food chains lead to high toxicity levels in animals [12]. Nevertheless, intensive agriculture needs continuous research for the development of new fertilizers and plagues controllers that care about environmental pollution and human health hazards. Biological methods would be a preferable alternative for controlling regional plants diseases using endogenous and domestic microorganisms.

The potential of *Trichoderma* strains as control plant pathogens embodies an economically attractive choice [13, 14, and 15]. In recent years, the search for *Trichoderma* isolates with a high antagonistic potential has increased [5, 2]. The application of strains with biological control capacities can help to reduce the input of chemical pesticides in agriculture, where it is necessary to introduce high levels of spores to the field. Therefore, agro industrial wastes can provide and economical and suitable source of substrates to produce significant amount of spores of *Trichoderma* [16, 17].

The primary aim of the present study was to isolate *Trichoderma* strains from soil samples of ecosystems without human impact in Misiones (Argentina). The native *Trichoderma* isolates were characterized by morphological observation and quick molecular identification. The strains were evaluated for their antagonist capacity against *Fusarium* sp., *Alternaria* spp. (strains 1 and 2), and *Botrytis* sp., which are phytopathogens that affect agriculture crops. Finally, agricultural wastes were assessed for their suitability as substrates for *Trichoderma* mass multiplication pursuing an economically and effective production methodology.

MATERIALS AND METHODS

Plant pathogens

Fusarium sp., *Alternaria* spp. (strains 1 and 2) and *Botrytis* sp., isolated from Argentina, were kindly provided by the Culture Collection of the Buenos Aires University (BAFC). These phytopathogens are the main responsible for plant diseases.

Soil sampling and fungal isolation

Soil samples were collected from "Ñacanguazu" creek in Gobernador Roca (GPS coordinates 27º06' S; 55º22' W), "Teyú Cuaré" Provincial Park in San Ignacio (GPS coordinates 27º16' S; 55º35' W) and "Profundidad" Provincial Park in Candelaria (GPS coordinates 27º33' S; 55º42' W), all in Misiones, Argentina. The soil samples were collected at 15 cm of depth in an area of 1 m^2 with 5 repetitions. The temperature and humidity of each collected place were recorded. The samples were immediately transported to the laboratory for pH and water content determination. The samples were diluted in sterile distilled water (1:1000 and 1:10000) and cultured using potato dextrose agar (PDA 3.9%), pH 6.5. After 10 days of incubation at 27±1°C, single colonies with morphology characteristic of *Trichoderma* sp. were selected for sub-culture and for further morphological and molecular identification. All strains were stored at -80°C in their respective liquid media with 15% glycerol.

DNA extraction and quick molecular identification

Fungal genomic DNA was extracted following a modified method described by Doyle [18]. Mycelia for DNA extraction were grown in liquid cultures at 27±1°C in malt extract broth (MEB) for 3-5 days in the dark. Mycelia was filtered and washed with 0.1 M Tris-HCl (pH 8) and 0.02 M EDTA (pH 8). DNA extraction was carried out with buffer solution (0.1 M Tris-HCl pH 8, 1.5 M NaCl, 0.05 M EDTA pH 8) at 60ºC, containing 0.1 mg/ml Proteinase K, 0.01 M β-mercaptoethanol and 2% SDS. DNA was purified with chloroform: isoamilyc alcohol (24:1) and 3M potassium acetate and then was precipitated with isopropyl alcohol. Pure DNAs extracted from the isolates were amplified at the ITS region and sequenced for phylogenetic analyses.

ITS1-5.8S-ITS2 region sequences of the ribosomal gene were used for fungi classification as described previously [19]. For the amplification, reaction primers ITS1 5' TTCGTAGGTGAACCTGCGG and ITS4 5' TCCTCCGCTTATTGATATGC [20] were used. The amplification reactions were prepared in a final volume of 20 µL containing 1X KCl buffer, 2.5 mM MgCl$_2$, 200 µM dNTPs for each 10 pmol primer [20], 0.5 U of Taq polymerase and template 5 ng/µl DNA. PCR amplification conditions were 94ºC for 40 s, 50ºC for 40 s and 72ºC for 40 s for 35 cycles, with 10 min extension at 72ºC used for the final cycle. Agarose gels at 1% and 2% were carried out to visualize genomic DNA and PCR products, respectively. The purification and sequencing procedures were performed by Macrogen Korea.

The ITS gene sequences of the isolated strains were compared with those deposited in the National Center for Biotechnology Information Database (NCBI, www.ncbi.nlm.nih.gov) with the special alignment search tool TrichoKey 2.0 and TrichoBLAST 2.0 to confirm *Trichoderma*

species [19] and with Database Fungal barcoding (www. fungalbarcoding.org/). The isolates had significant hits to the genus that owed the lowest e-value in the results of BLAST. Alignments of nucleotide sequences were carried out with the Clustal W software [21]. To allow an appropriate phylogenetic analyses, sequences of another strains of *Trichoderma* were included in the present study (accession numbers: JQ040311, EU280132, EU280136, EU280105, KP898755, FJ430784, JX125615, HQ596981, KF294838, KJ767092, KC478546, HQ637329, HQ260623, EU280095, DQ200259, HM461859, KC582841, FJ459964, JN943376, JN943375, JN943374, AY737767, FJ860752, AF275322, AY737762, HM142362, GU934533, JX069200, JX069201, EU280087, KC884785, KC561076, JX908732). The parsimony analyses were performed with Bootstrap methods [22] included 1000 replications. In the analyses, *Hypomyces subiculosus* (EU280093) were used as an out-group.

Fungal inhibition assays

Nine cm Petri plates containing potato dextrose agar (PDA) were inoculated with 7 mm mycelial discs of either *Fusarium* sp., *Alternaria* sp. or *Botrytis* sp., and *Trichoderma* isolates 10 mm away from the edge of the plate opposite to each other. Plates inoculated with the *Trichoderma* strains and pathogens strains alone served as control. Plates were incubated at 27±1°C for *Alternaria* sp. and *Fusarium* sp. and 14±1°C for *Botrytis* sp. Three replicate plates were done for each treatment. The radial growth was measured progressively. The efficacy of the antagonist in inhibiting the pathogen growth was evaluated quantitatively by the inhibition grade formula. The percentage of growth inhibition was calculated using the equation RI=100 x (R2 - R1)/R2 when the contact started, where RI was the percentage of reduction in mycelial growth, R1 was the averaged growth of pathogen in treated plates and R2 was the averaged growth of pathogen in control plates [23]. An effective antagonist strain is capable of inhibiting 50% or more the pathogen growth. The antagonism index was calculated after ten days of the starting day with Bell scale modified by Calistru [24, 25]: 4 corresponded to 100% *Trichoderma* sp. coverage on the phytopathogen and 0 corresponded to 100% phytopathogen coverage over *Trichoderma* strains. An index of 3 or 4 it is considered an effective antagonist strain.

Culture substrates of *Trichoderma* strains for mass production

The best sporulation condition of *T. harzianum* LBM 096 using agro industrial wastes as solid substrate for microbial cultivation was assayed. Rice was used as control. The substrates consisted of sawdust, rice polishings, rice flour and rice husk with different moisture content depen-

ding on the treatment (Table 1). As primary inoculum the strain was grown on 2 g/ml rice substrate. Fermentation assays were carried out without external nutrient addition. All substrates were autoclaved for 15 min at 121°C, inoculated with $3x10^8$ spores and incubated at 27±1°C in natural light for 15 days. Spores were removed by washing with distilled water containing 0.5% Tween 20 and were counted following the Neubauer method.

Table 1: Media formulations with different local agro industrial wastes.

Treatment	Substrate					Water
	Sawdust	Rice polishings	Rice flour	Rice husk	Rice	
1	40 g	-	-	-	-	20 ml
2	20 g	20 g	-	-	-	20 ml
3	20 g	-	20 g	-	-	20 ml
4	-	-	-	20 g	-	20 ml
5	-	10 g	-	10 g	-	20 ml
6	-	-	10 g	10 g	-	20 ml
7	-	-	-	-	40 g	20 ml

Statistical analysis

The data obtained in all experiments were subjected to analysis of variance (ANOVA) with Turkey's test using the SPSS software, version 22.0 (IBM Corp, Armonk, NY, USA), at a significance level of 5%.

RESULTS

Soil sampling and fungal isolation

Out of 20 colonies per plate obtained from soil samples, 15 fungal isolates were selected as probable *Trichoderma* strains (Table 2). Aerial mycelium of the colonies was initially thick and whitish. When the sporulation started, the colony turned green and the periphery of the colonies remained white. At a microscopical level, we identified phialides with a flask-shaped formed at wide angles to the conidiophore, and with conidia clustered together at the end of each phialide.

Table 2: Locations and soil conditions of fungal isolates

Soil Sample	Location	Isolate	RH %	T °C	pH	WC %
1	Ñancanguazú stream	LBM 090	70	17.8	7.60	36.4
2	Ñancanguazú stream	LBM 091 LBM 092 LBM 093 LBM 094	70	17.1	7.28	44.6
3	TeyúCuaré	LBM 103	65	17.2	7.86	45.8
4	TeyúCuaré	LBM 104	66	16.5	7.56	37.8
5	Profundidad	LBM 095 LBM 096 LBM 097 LBM 098 LBM 099 LBM 100 LBM 101 LBM 102	55	15.0	7.60	36.9

Note: RH: relative humidity, WC: water content.

DNA extraction and quick molecular identification

We were able to accurately classify at genus level the isolated *Trichoderma* strains by applying morphological criteria.

The ITS1-5.8S-ITS2 gene sequences of the 15 isolates were compared with those sequences deposited in the three database utilized, indicating that they had significant hits to the genera *Trichoderma.* Seven isolates were identified as *Trichoderma harzianum* (LBM094, accession number of NCBI: JX069200, LBM096, accession number of NCBI: JX069196, LBM097, accession number of NCBI: JX069197, LBM100, accession number of NCBI: JX069198, LBM101, accession number of NCBI: JX069199, LBM103, accession number of NCBI: JX069201, LBM104, accession number of NCBI: JX069195), six as *Trichoderma koningiopsis* (LBM090, accession number of NCBI: JX069205, LBM091, accession number of NCBI: JX069206, LBM092, accession number of NCBI: JX069207, LBM098, accession number of NCBI: JX069202, LBM099, accession number of NCBI: JX069203, LBM102, accession number of NCBI: JX069204), one as *Trichoderma brevicompactum* (LBM095, accession number of NCBI: JX069193), and

one as *Trichoderma pleuroticola* (LBM093, accession number of NCBI: JX069194). All strains, in all database consulted, had 97% to 100% of similarity with the reference sequence of each species.

A phylogenetic tree using the fifteen *Trichoderma* spp. isolated ITS sequences [19] was constructed based on parsimony analyses (Figure 1).

The phylogenetic analysis revealed close positioning of seven isolates of *T. harzianum* (LBM094, LBM096, LBM097, LBM100, LBM101, LBM103, LBM104) and *T. pleuroticola* LBM093 in a closely related group, in concordance with actual taxonomic classification of clade Harzianum, Pachybasium Section (accessible at http://www.isth.info/biodiversity/index.php). The six isolates corresponding to *T. koningiopsis* (LBM090, LBM091, LBM092, LBM098, LBM099, LBM102) revealed close related positioning in the same group, in Trichoderma Section. *T. koningiopsis* LBM091 and LBM092 corresponded to the same strain because they were no genetic distance between them and were isolated from the same place (Ñancanguazú creek). Finally, *T. brevicompactum* LBM 095 showed high genetic distance to the other strains and belongs to Lutea clade, Lone Lineages Section (Figure 1).
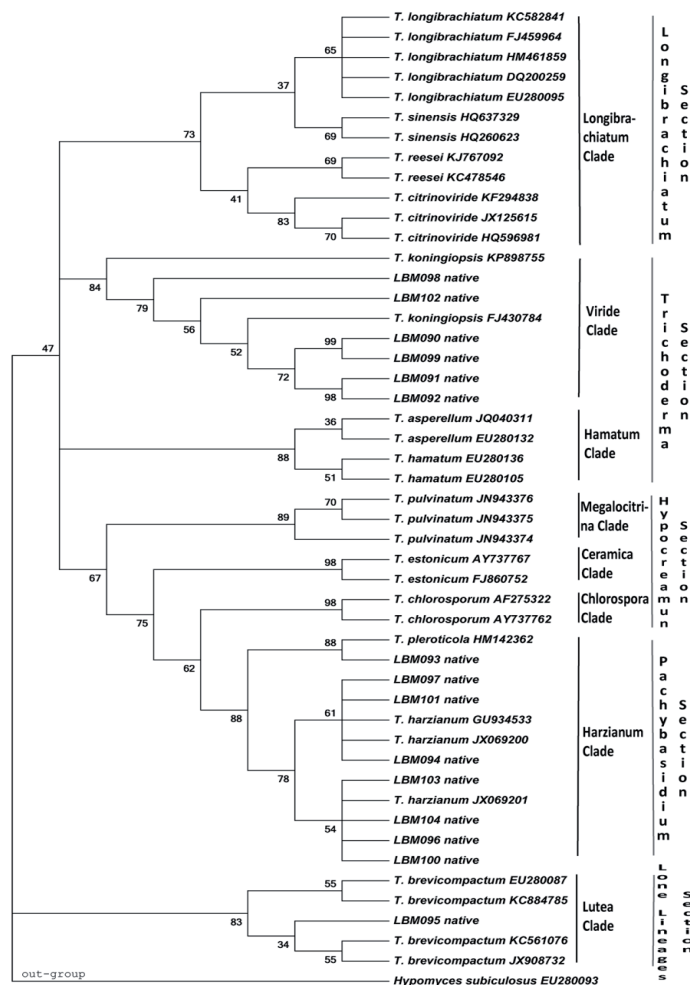

Figure 1: Parsimony analysis based on ITS sequences from all isolates of Trichoderma. Group support with 1000 Bootstrap.

Fungal inhibition assays

On dual confronted cultures, *Trichoderma* strains reached *Fusarium* sp. and *Alternaria* sp. (strain 1) in 3 days, *Alternaria* sp. (strain 2) in 4 days and *Botrytis* sp. in 10 days (Table 3). The strains *T.a koningiopsis* LBM 090 and LBM 091, LBM 098, LBM 092, *T. pleuroticola* LBM 097, *T. harzianum* LBM 096 and *Trichoderma koningiopsis* presented %RI higher than 50% indicating that strains are effective antagonists. Among the isolates, *T. koningiopsis* LBM 090 and *T. koningiopsis* LBM 098 showed the highest percentage of growth inhibition (60-70%) against *Botrytis* sp. (Table 3).

From all strains studied, inhibition by the *Trichoderma* strains in dual cultures did not show statistically significant differences with *Alternaria* sp. (strain 1), *Alternaria* sp. (strain 2) and *Fusarium* sp. In contrast, *Trichoderma* strains showed significant differences with *Botrytis* sp. *Trichoderma koningiopsis* LBM090 showed the best %RI in dual confronted assays towards the four phytophatogens strains studied (Table 3). Based on the result obtained with Bell scale modified, all strains resulted effective antagonists with a value of 3 and 4 in this scale indicating 100% coverage of the pathogen colonies. *Trichoderma koningiopsis* LBM090, LBM091 and LBM098 presented a value of 4 to all strains of pathogens. These strains exerted antagonist and parasitism capacities against regional phytopathogens and would be potential biological controls.

Table 3: Mycelial growth inhibition percentage (%RI) of *Trichoderma* strains versus phytopathogenic fungal strains.

	Alternaria sp. (strain 1)	*Alternaria* sp. (strain 2)	*Fusarium* sp.	*Botrytis* sp.
	%RI on day 3	%RI on day 4	%RI on day 3	%RI on day 10
T. koningiopsis LBM 090	36.51 ± 5.50a	39.73 ± 5.48a	51.44 ± 1.87a	63.11 ± 3.98a
T. koningiopsis LBM 091	28.57 ± 4.76a	15.98 ± 3.16a	46.63 ± 5.46a	49.67 ± 1.65ab
T. koningiopsis LBM 092	28.57 ± 4.76a	28.77 ± 5.48a	46.63 ± 1.52a	45.19 ± 7.80ab
T. koningiopsis LBM 098	33.33 ± 9.52a	34.25 ± 7.25a	51.44 ± 5.72a	58.89 ± 5.47ab
T. koningiopsis LBM 099	25.40 ± 5.50a	30.60 ± 6.33a	41.38 ± 5.46a	43.08 ± 2.74ab
T. koningiopsis LBM 102	16.66 ± 3.37a	30.59 ± 4.18a	45.76 ± 1.52a	36.76 ± 3.16ab
T. harzianum LBM 094	17.86 ± 1.69a	23.29 ± 5.48a	39.63 ± 6.94a	nd
T. harzianum LBM 096	30.16 ± 7.27a	26.94 ± 8.37a	45.76 ± 6.06a	45.19 ± 2.41ab
T. harzianum LBM 097	30.16 ± 7.27a	32.74 ± 7.85a	42.26 ± 10.50a	45.19 ± 5.08ab
T. harzianum LBM 101	18.25 ± 3.64a	28.77 ± 5.48a	40.51 ± 1.52a	37.55 ± 1.12ab
T. harzianum LBM 100	20.63 ± 2.75a	24.20 ± 6.90a	40.07 ± 4.97a	32.02 ± 7.91b
T. harzianum LBM 103	25.40 ± 5.50a	23.30 ± 0.01a	43.57 ± 3.47a	42.55 ± 1.82ab
T. harzianum LBM 104	26.42 ± 3.55a	26.30 ± 1.51a	42.67 ± 4.74a	39.65 ± 3.21ab
T. brevicompactum LBM 095	29.36 ± 3.64a	25.11 ± 6.33a	37.88 ± 1.52a	39.92 ± 5.47ab
T. pleuroticola LBM 093	25.40 ± 2.75a	23.29 ± 7.75a	43.13 ± 1.52a	48.88 ± 7.80ab

Note: Means followed by the same letter in each column do not differ significantly by Tukey test at 5% probability. nd: not determined.

Culture substrates of *Trichoderma* strains for mass production

The suitable condition for mass multiplication of *T. harzianum* LBM096 was evaluated. Among the substrates evaluated, the mix of rice husk and rice polishing yielded the highest sporulation (1.96×10^{10} spores/g substrate) followed by rice husk and rice flour (1.45×10^{10} spores/g substrate), sawdust and rice polishing (1.12×10^{10} spores/g substrate) and sawdust and rice flour (6.4×10^{9} spores/g substrate). Results indicated that the best spore production was found in substrates with rice flour and rice polishing. Significantly lower spore concentration was recorded in 1, 3, 4 and control treatments (Figure 2).
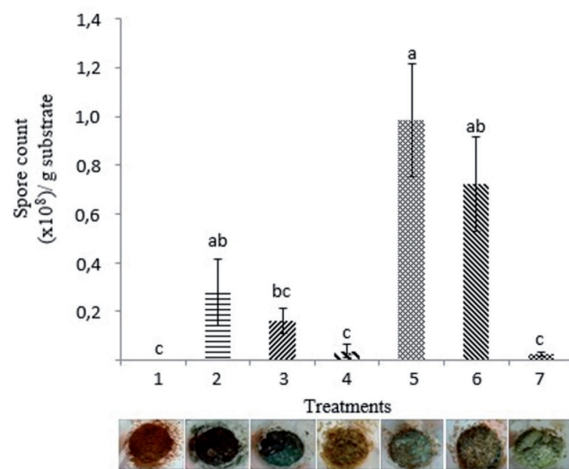

Figure 2: Evaluation of substrates for mass production. Identical number means no statistically significant differences between these treatments (P > 0.05). 1: Sawdust; 2: Sawdust and Rice polishings; 3: Sawdust and Rice flour; 4: Rice husk; 5: Rice polishings and Rice husk; 6: Rice flour and Rice husk; 7: Rice.

DISCUSSION

The fifteen *Trichoderma* strains were isolated from low human impacted ecosystems because the expected diversity, as it is documented by other authors [26, 27, 28].

The correct identification at species level is highly desirable because some of them can be possible risks to human health [29]. Systematic studies of microorganisms were based almost exclusively on morphological criteria (classical microbiological classification). Currently, the tools used in systematic studies have increased, and not only the morphological criteria, but also molecular criteria are considered. However, the number of tools used in systematic studies has increased. Nowadays, both morphological and molecular criteria need to be taken into account [30, 31]. Since the primary aim of the present study was to isolate *Trichoderma* strains from soil samples from low human impacted ecosystems, in this work it was done a rough molecular identification of the *Trichoderma* strains using only ITS Barcodes. We considered the classical microbiological classification by morphological criteria and

molecular techniques by amplification of ITS1, 5.8S and ITS2 regions. The ITS1-5.8S-ITS2-28S regions can have nucleotide variations since their transcripts are excised from the final rRNA fragments. Therefore, the ITS sequence includes both ITS1 and ITS2, which are separated by the conserved short 5.8S rRNA. They are usually used to infer phylogenetic relationships of closely related species as well as to assess the variability of a population, e.g. of geographically distant isolate (ecotypes) and recommended by Druzhinina et al. [19] as Hypocrea/Trichoderma barcode. These regions are appropriate for detecting differences between conspecific individuals and hence, are potentially useful markers to study the relationships of populations and closely related species in fungal, plant, and animal taxa due to their relatively rapid evolutionary rates [32, 33]. The ITS was chosen as the official barcode for fungi by a consortium of mycologists and it is among the markers with highest probability of correct identification for a very broad group of sampled fungi [34, 35]. Recently, there are many researches that used tef-1 and RPB2 as complementary barcodes for *Trichoderma* species identifications [36].

We isolated 15 native strains of *Trichoderma* genus from Misiones, Argentina to study their antagonist capacity. *T. koningiopsis* LBM 090 was the most promissory strain; however, *T. koningiopsis* LBM 090, LBM 091 and LBM 098 showed some interesting aspects as potential biological control agents. Two strains of *T. koningiopsis* (LBM090 and LB098) showed the best results in the antagonism tests. However, using qualitative analyses, all strains were capable to reduce and invade more than 75% of the phytopatogen fungi growth after ten days. These results indicate the potential of antagonistic strain to reduce diseases in field conditions. Larralde-Corona *et al.* [37], selected new *T. koningiopsis* strains as biological control agents to sorghum phytopathogens. There are other reports showing the inhibitory effect of other *Trichoderma* species on the same phytopathogens used in this work. *T. harzianum* on *Alternaria alternata*, for tobacco [1]; *T. harzianum* on *Fusarim oxysporum* with similar %RI [38]; *T. harzianum* on *Fusarim solani* [39]; *T. hamatum* and *T. atroviride* for *Pinus radiata* [40], *Trichoderma asperellum* strain CCTCC-RW0014 showed to have good biocontrol potential with disease reduction of 71.67% against *Fusarium oxysporum f. sp. cucumerinum* [41]. Pugliese *et al.* [42] selected antagonistic fungi from compost as a promising strategy for the development of new biological control agents against soil-borne pathogens.

One important aspect to biotechnological prospection is a high production of spores in an adequate substrate. Forestry is one of the principal activities of Misiones state and sawdust reuse is a government priority. Here, we demonstrated the convenience of sawdust as a substrate for spores production at level superior of 10^8, in concordance with systems proposed for other substrates [43, 44, 16]. Interestingly, our system with sawdust produced more spores than the sawdust system proposed by Kumar and Palakshappa [45].

Conclusions

In conclusion, 15 strains were isolated in natural soils of Misiones province. Several candidate strains were identified to act against *Alternaria* sp., *Fusarium* sp. and *Botrytis* sp. The formulation of rice husk and rice polishing resulted as the best suitable combination of substrates to increase the mass production. Our results are the starting point for future studies on the utilization of native strains with antagonist properties in our region. It is still needed to study deeply the biological control mechanisms in field experiments with these strains.

Acknowledgements

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