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Isolation and characterization of native *Trichoderma* spp. and phytopathogenic fungi in Paraguay

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Introduction

Agriculture is a fundamental activity in Paraguay, reaching at least 41% of the national economy, indicating a very high participation of primary productive activities in the daily life of society (Ferreira y Vázquez 2015). The sector confronts problems that lead to a reduction of production and therefore the economy. One of the major problems is the disease of plants, which are caused by various pathogens known as phytopathogens, among which bacteria, viruses and fungi can be mentioned (Monte 2001). Agrochemicals are the main tool to combat these diseases and the indiscriminate use of these products has led to the development of resistance to fungicides as well as to the severe consequences to the environment and to humans (Ávila et al. 2014).

In order to reduce these effects, the current trend has been to rationalize the use of chemical products and develop new control alternatives through the use of biological agents (Ávila et al. 2014). The species of the genus *Trichoderma* are the most used for the biocontrol of diseases of plants produced by fungi, due to their characteristics of having mechanisms of competition for nutrients, mycoparasitism, and production of antibiosis (Weindling 1934), antifungal metabolites and hydrolytic enzymes to deal with the aggressor (Ezziyyani et al. 2004). Using strains of biocontrol that are native allows to obtain greater possibilities of success, these strains can be better adapted to the environmental conditions in which they are to introduce, in front of formulated products introduced that at the moment of their application may or may not be efficient, being able also affect the ecological balance. (López-Valenzuela et al. 2015, Moya et al. 2014).

The objective of the work was to isolate and characterize morphologically native strains of *Trichoderma* spp. and phytopathogens from different growing regions of Paraguay, as well as establish the methodological conditions for the molecular characterization of native *Trichoderma* isolates.

Methodology

Samples of agricultural soil, rhizosphere, seeds and plant material collected from different areas and crops of Paraguay were used. Each material was georeferenced through a Global Positioning System (GPS) and was transported to the laboratory for analysis.

For the observational descriptive procedure for obtaining *Trichoderma* spp. and phytopathogenic fungi, we used the Fernández (1993) soil dilution method and the morphological identification codes of Barnett and Hunter (1998) for selection. Monosporic cultures were identified by sequential alphanumeric codes. For the analysis by Polymerase Chain Reaction (PCR) technique the DNA was extracted by the hexadecyltrimethylammonium bromide (CTAB) method of the isolates FCQ13 to FCQ22 and FCQ41, a fragment of the tef1 gene was amplified with EF1-728F / tef1rev and with ITS1 / ITS4 the rDNAs ITS1-5.8S-ITS2 regions, three strains identified at the species level were used as reference for the molecular characterization, *Trichoderma harzianum* T34 (CECT2413), *Trichoderma brevicompactum* IBT40841 y *Trichoderma arundinaceum* IBT4083, obtained through a collaboration with the University of Cadiz, Spain.

Results and discussion

A total of 33 monosporic isolates of *Trichoderma* spp. and phytopathogenic fungi were obtained. A total of 24 correspond to the genus *Trichoderma*, and 9 correspond to phytopathogenic fungi, among which isolates of *Macrophomina phaseolina*, *Fusarium* spp., *Rhizoctonia* spp., *Colletotrichum* spp. and *Sclerotinia* spp. Table 1 shows the Departments, cities, GPS data and the type of crop from which they were isolated. The isolation of fungi such as *Trichoderma* and phytopathogens was carried out from several types of substrates, in this respect, it is described that the fungi of the genus *Trichoderma* naturally inhabit a variable number of agricultural soils, with abundant organic

Table 1. Description of the *Trichoderma* spp. and phytopatogens obtained from the different regions and crops in Paraguay.

Coordinates	Place of colletion	District	Typeof sample	Culture	Isolated fungus	Isolated code
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP*	soil	Tomato	*Trichoderma* spp.	FCQ13-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ14-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ15-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ16-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ17-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ18-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ19-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ20-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ21-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Tomato	*Trichoderma* spp.	FCQ22-TENTO16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	soil	Pepper & onion	*Trichoderma* spp.	FCQ28-TENPI16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	plant material	Pepper & onion	*Colletrtrichum* spp.	FCQ29-COENCE16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	Rhizosphere	Pepper	*Trichoderma* spp.	FCQ23-TENPI16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	Rhizosphere	Pepper	*Trichoderma* spp.	FCQ24--TENPI16
-26, 452996898442507 ;'-55,26637142524123	Itapúa	Tomas RP	Rhizosphere	Pepper	*Fusarium* spp.	FCQ25-FUENPI16
-25,3801242; -57,4851362	Central	Capiatá	soil	Pepper	*Trichoderma* spp.	FCQ30-TCEPI16
-25,3801242; -57,4851362	Central	Capiatá	soil	Pepper	*Trichoderma* spp.	FCQ32-TCEPI16
-25,3801242; -57,4851362	Central	Capiatá	soil	Pepper	*Fusarium* spp.	FCQ33-FUCEPI16
-25,3801242; -57,4851362	Central	Capiatá	soil	Pepper	*Fusarium* spp.	FCQ34-FUCEPI16
-25,3801242; -57,4851362	Central	Capiatá	Rhizosphere	Pepper	*Rhizoctonia* spp.	FCQ35-RHICEPI16
-25,3593139; -57,0441465	Cordillera	Barrero	soil	Pepper	*Trichoderma* spp.	FCQ41-TCOPI16
-25,3593139; -57,0441465	Cordillera	Barrero	soil	Pepper	*Trichoderma* spp.	FCQ42-TCOPI16
-25,3593139; -57,0441465	Cordillera	Barrero	soil	Pepper	*Trichoderma* spp.	FCQ43-TCOPI16
-25, 891268; -55, 355942	Alto Paraná	Hernandarias	soil	Corn	*Trichoderma* spp.	FCQ36-TITAMA16
-25, 891268; -55, 355942	Alto Paraná	Hernandarias	soil	Soy	*Trichoderma* spp.	FCQ37-TITASO16
-25, 891268; -55, 355942	Alto Paraná	Hernandarias	soil	Soy	*Trichoderma* spp.	FCQ38-TITASO16
n.d.	San Pedro	Chore	seeds	Sesame	*Macrophomina*	FCQ26- MASPSE16
n.d.	San Pedro	Chore	seeds	Sesame	*Macrophmina*	FCQ27- MASPSE16
n.d.	Itapuá	Edelira	stubble	Soy	*Macrophomina*	FCQ39-MAITASO16
n.d.	Cordillera	Cabañas	plant material	Pepper	*Sclerotinia* spp.	FCQ40-SCSCCOPI16
n.d.	Cordillera	Caacupé	soil	Stevia	*Trichoderma* spp.	FCQ44-TCOKH16
n.d.	Cordillera	Caacupé	Formulated /soil	Commercial product	*Trichoderma* spp.	FCQ45-TCO16
n.d.	Cordillera	Caacupé	soil	Ornamental	*Trichoderma* spp.	FCQ46-TCOOR16

*Tomás RP: Tomás Romero Pereira, n.d.: not determinated

matter in decomposition and high root densities (Agamez et al. 2008).

The amplification of the ITS regions (ITS1, ITS2 and the 5.8S gene) of the rDNA for the 11 isolates, isolates FCQ13 to FCQ22 and FCQ41 (Figure 1), generated products of different sizes and the amplification products of a fragment of the tef1 gene also showed a variability between the sizes, these results are attributed to the variability characteristics of the sequences of both amplified regions, and similar to the results described by Sadfi-Zouaoui et al. (2009). However, it was not possible to amplify the sequence of the tef1 gene corresponding to strain FCQ41 despite having been repeated multiple times in independent preparations. It is important to note

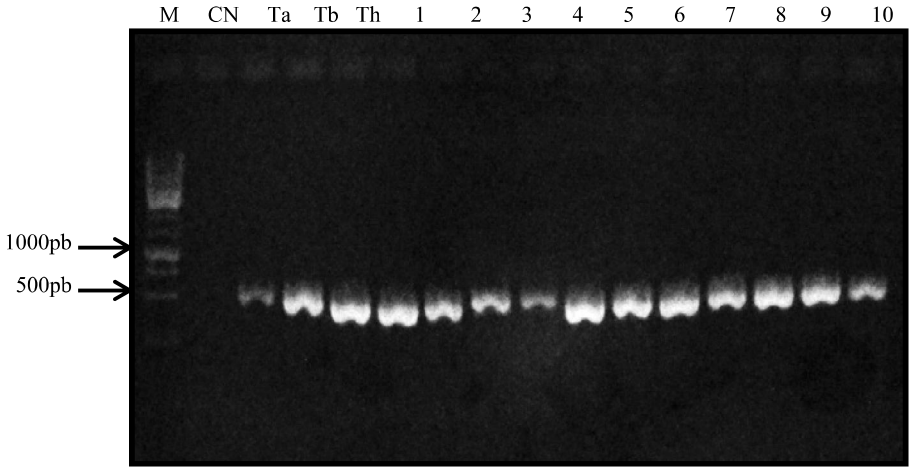

Figure 1. Agarose gel electrophoresis (1% w/v) of PCR products from ITS (ITS1-5.8S-IT2) regions of *Trichoderma* strains. M: Size marker (1kb), CN: Negative control. Reference strains, Ta: (*Trichoderma arundinaceum*), Tb: (*Trichoderma brevicompactum*), Th: (*Trichoderma harzianum*). 1 to 10: FCQ13 to FCQ22, 11: FCQ41

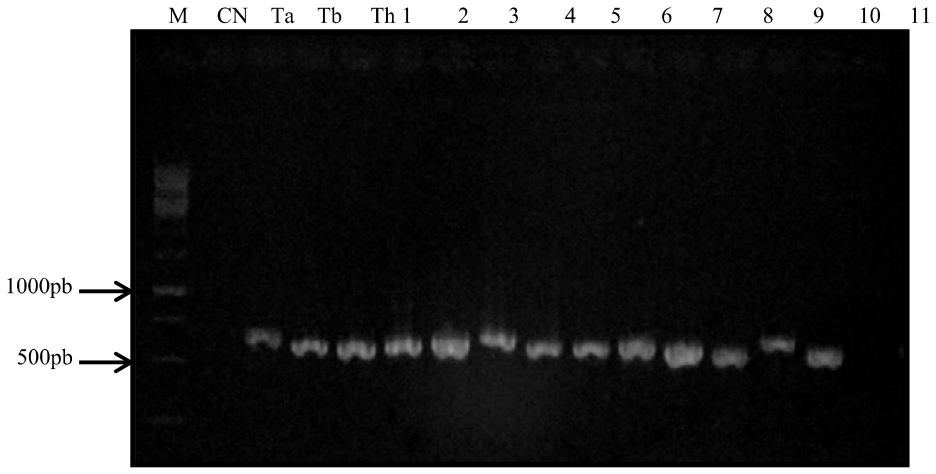

Figure 2. Agarose gel electrophoresis (1% w/v) of PCR products from tef1 regions of *Trichoderma* strains. M: Size marker (1kb), CN: Negative control. Reference strains, Ta: (*Trichoderma arundinaceum*), Tb: (*Trichoderma brevicompactum*), Th: (*Trichoderma harzianum*). 1 to 10: FCQ13 to FCQ22, 11: FCQ41.

that this strain was previously amplified with STS, so a lack of DNA is ruled out in the original sample, this result is not attributed to the problems during the procedure performed in the experiment, which allows to propose that the lack of amplification may be due to the fact that the primers for tef1 are not hybridizing properly for that isolate and thus their amplification is not achieved which could be due to possible variations in the sequence of the hybridization site of the primers. These results reinforce the need to use more than one marker for a more specific identification study of this group of fungi.

Conclusion

With the isolation methodology and subsequent morphological characterization, 24 strains of *Trichoderma* spp. with distinguishable morphological characteristics and 9 phytopathogenic fungi of Paraguay were obtained. The conditions that were analyzed by the ITS regions and a fragment of the tef1 gene were able to obtain the amplification of the regions with the expected product sizes.

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