



Isolation and molecular characterization of native *Trichoderma* species in sites with different management practices in Chilpancingo, Guerrero, Mexico

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ABSTRACT

Background/Objective. The genus *Trichoderma* comprises fungi widely recognized for their capacity as biological control agents and plant growth promoters; however, information on their molecular diversity in agricultural systems in Guerrero, Mexico, is limited. The objective of this study was to isolate and molecularly identify native *Trichoderma* species present in different sites within the municipality of Chilpancingo de los Bravo, Guerrero, and to analyze their distribution in relation to management conditions.

Experimental development. During August and September 2025, 125 traps with sterile rice were installed at four sampling points: Palo Blanco (Agroecological system), the Botanical Garden of the Universidad Autónoma de Guerrero (Plant conservation), and two areas of Petaquillas (Unmanaged natural vegetation). Isolates were purified by monospore culture, morphologically characterized, and identified by ITS region sequencing and maximum likelihood phylogenetic analysis with 1,000 bootstrap replicates.

Results. Seven *Trichoderma* isolates were obtained, recovered only from the Palo Blanco agroecological plot and the Botanical Garden of the Universidad Autónoma de Guerrero. Phylogenetic analysis identified *T. harzianum/Hypocrea lixii*, *T. asperellum*, and *T. longibrachiatum*. Representatives of *T. harzianum/H. lixii* and *T. longibrachiatum* were identified at Palo Blanco, while *T. harzianum/H. lixii* and *T. asperellum* were identified at the Botanical Garden. The *T. harzianum/H. lixii* complex was the predominant taxon, representing 57.1% of the isolates obtained.

Conclusion. Three *Trichoderma* taxa were identified, with the *T. harzianum/Hypocrea lixii* complex predominating (57.1%), present in Palo Blanco and the Botanical Garden. The results expand our knowledge of its diversity and distribution in Guerrero, although its biotechnological potential should be confirmed through functional evaluations.

Keywords: Phylogeny, Rhizosphere, Microbiota, Taxonomy, Soil

INTRODUCTION

The genus *Trichoderma* (*Hypocrea*, as the teleomorphic form) comprises a diverse group of filamentous fungi widely distributed in agricultural soils, organic residues, and the rhizosphere of numerous plant species (Kubiak *et al.*, 2023). In agroecosystems, these species are notable for their ability to act as biological control agents and plant growth promoters through mechanisms such as competition for space and nutrients, mycoparasitism, antibiosis, and the induction of defense responses in plants (Yao *et al.*, 2023; Ismaiel *et al.*, 2024). In addition, various species are capable of synthesizing phytohormones and promoting nutrient mobilization, thereby contributing to crop development and productivity (Michel-Aceve *et al.*, 2013; Illescas *et al.*, 2021; López-López *et al.*, 2023).

In the context of sustainable agriculture, interest in beneficial soil microorganisms has increased owing to their potential to reduce dependence on agrochemicals and promote biological processes associated with soil health (Torres-De la Cruz *et al.*, 2015; Cucu *et al.*, 2025). Several studies have documented that edaphic conditions, soil management, and organic matter content influence the composition of microbial communities, including native *Trichoderma* populations (Gupta *et al.*, 2022; Kinsbergen *et al.*, 2025). In this regard, agroecologically managed systems have gained relevance because they promote practices aimed at conserving biodiversity, recycling nutrients, and maintaining soil quality through strategies such as crop diversification, incorporation of organic matter, reduced agrochemical use, and conservation of the soil microbiota (Wezel *et al.*, 2020; Banerjee and van der Heijden, 2023). These practices can foster more stable environments for the establishment and persistence of beneficial microorganisms, including *Trichoderma* species, which participate in processes related to soil health and functionality (Poveda, 2021). Accordingly, agroecological systems represent environments of interest for the search, isolation, and characterization of native species with potential biotechnological applications. In this context, the study of the local diversity of these microorganisms constitutes a relevant strategy for identifying isolates adapted to specific management conditions and with potential value for their use in sustainable agricultural systems (Zin and Badaluddin, 2020; Natsiopoulou *et al.*, 2024; Shao *et al.*, 2025).

Identification of *Trichoderma* species based solely on morphological characteristics presents limitations owing to the phenotypic similarity among species and the plasticity of their reproductive structures (Surma *et al.*, 2025). Accordingly, the internal transcribed spacer (ITS) region of ribosomal DNA constitutes one of the most widely used molecular markers for fungal identification and preliminary taxon delimitation within this genus. However, recent studies recommend complementing its use with higher-resolution markers, such as translation elongation factor 1- α (TEF1- α) and RNA polymerase II subunit 2 (RPB2), particularly for discriminating closely related species and taxonomic complexes within the genus (García-Núñez *et al.*, 2017; Cai and Druzhinina, 2021).

In Mexico, studies exist on the molecular diversity and biotechnological potential of *Trichoderma*; however, in Guerrero this information is limited, particularly in local agricultural systems characterized by the production of maize, beans, agave, and other crops managed under traditional or agroecological schemes (Ahedo-Quero *et al.*, 2024; Matas-Baca *et al.*, 2023). The objective of the present study was to isolate and molecularly identify native *Trichoderma* species present at different sites in the municipality of Chilpancingo de los Bravo, Guerrero, and to analyze their distribution as a function of management conditions, under the assumption that environments with greater plant

diversity, organic matter input, and lower chemical disturbance favor greater recovery of native isolates.

EXPERIMENTAL DEVELOPMENT

Fungal sampling and isolation. *Trichoderma* isolates were obtained using rice-based baiting traps, following the methodology described by Vega-Acosta (2016). Prior to use, the rice was subjected to three consecutive washes with water to remove excess starch and then allowed to air-dry at room temperature. The substrate was not cooked and was sterilized directly by autoclaving at 121 °C for 20 min before being used in the traps. Disposable cups covered with mesh fabric secured with rubber bands over the opening were used for this purpose, each containing 30 g of rice. The traps were placed at three sites in the municipality of Chilpancingo, Guerrero, during August and September 2025, arranged in a zigzag pattern, buried at a depth of 30 cm, with a spacing of 3 m between sampling points. A total of 25 traps were installed in the Botanical Garden of the Universidad Autónoma de Guerrero, 50 in the locality of Palo Blanco, and 50 in the locality of Petaquillas, for a total of 125 traps distributed across the study sites.

The evaluated sites differed in terms of land use and soil management. The locality of Palo Blanco corresponded to a plot managed under agroecological principles, characterized by a polyculture system integrating agave, maize, beans, squash, fruit trees, and timber species. Management of the system included the application of compost as an organic fertilizer, reduced agrochemical use through bioinoculants, minimum tillage practices, and periodic monitoring of soil conditions. The Botanical Garden of the Universidad Autónoma de Guerrero, in turn, corresponds to an area dedicated to the conservation and maintenance of various ornamental, forestry, and native species, managed through physical control of unwanted vegetation while avoiding agrochemical use in order to favor the conservation of the native soil microbiota. Water was supplied by manual irrigation. Additionally, sampling points were evaluated in the locality of Petaquillas, corresponding to areas with spontaneous vegetation and natural cover, with no recent history of cultivation, agricultural input application, or agronomic management practices; these were therefore considered zones without productive management. After 10 days, the traps were recovered and transported to the Laboratory of Molecular Microbiology and Environmental Biotechnology of the Facultad de Ciencias Químico-Biológicas for processing.

Cultural and morphological characterization of the isolates. Fungal isolation was performed by recovering the mycelium developed on the trap mesh, followed by transfer via puncture to Petri dishes containing potato dextrose agar medium (PDA; MCD LAB®). Cultures were incubated at room temperature for seven days and subsequently purified by monospore culture until axenic isolates were obtained (García-Latorre and Poblaciones, 2024). As a reference for morphological and molecular analyses, an isolate from the commercial product Trichozyme® was included and considered as commercial control. Cultural and morphological characterization was performed using seven-day monospore cultures grown on PDA medium, through cultural and microscopic evaluation of the reproductive structures.

Molecular characterization and phylogenetic analysis. Genomic DNA was obtained from fresh mycelium using a Quick-DNA Fungal/Bacterial Miniprep kit (Zymo

Research®). The ITS region (ITS1-5.8S-ITS2) of ribosomal DNA was amplified by PCR using the primers SR6R (5'-AAGTAGAAGTCGTAACAAGG-3') and LR1 (5'-GGTTGGTTTCTTTTCCT-3'), yielding amplicons of approximately 700 bp (Guigón-López *et al.*, 2010). The amplified products were purified and sequenced in both directions at the Instituto de Biotecnología of the Universidad Nacional Autónoma de México. The resulting sequences were edited and assembled in BioEdit v7.0.9, while preliminary identification was performed using BLAST against the NCBI GenBank database (Altschul *et al.*, 1990). A phylogenetic tree was subsequently constructed using the Maximum Likelihood method with 1000 bootstrap replicates in MEGA 7.0 (Madeira *et al.*, 2024), using *Aspergillus niger* (LC632396.1) as the outgroup.

Diversity of *Trichoderma* by sampling site. Trapping yielded seven native isolates associated with the genus *Trichoderma*, obtained from only two of the four sites evaluated (Table 1). The presence of the genus was recorded in the Botanical Garden of the Universidad Autónoma de Guerrero and in the locality of Palo Blanco, whereas no growth compatible with *Trichoderma* was observed at either of the two sites corresponding to Petaquillas. In Palo Blanco, five isolates (AT5–AT9) were recovered from 50 traps, while in the Botanical Garden, two isolates (AT1 and AT2) were obtained from a total of 25 traps. This distribution suggests spatial variability in the recovery of the genus, possibly associated with environmental and edaphic factors, as well as differences in local soil management. It has been documented that the abundance of *Trichoderma* can vary according to temperature, humidity, sampling timing, and the physicochemical characteristics of the soil (Michel-Aceves *et al.*, 2009; López-López *et al.*, 2022).

Table 1. Location of sampling sites and *Trichoderma* isolates recovered according to site management type.

Sampling site	Management type	Latitude (N)	Longitude	Traps	Recovered isolates
UAGro Botanical Garden	Plant conservation	17°32'17.3''	-99°29'38.7''	25	AT1, AT2
Palo Blanco	Agroecological system	17°22'32.412''	-99°28'52.3''	50	AT5, AT6, AT7, AT8, AT9
Petaquillas	Unmanaged natural vegetation	17°29'04.4''	-99°27'26.5''	25	-
		17°29'11.9''	-99°28'02.8''	25	-
Commercial control (Trichozyme®)	Commercial product	-	-	-	TC

Cultural and morphological characterization. The colonies exhibited rapid radial growth, completely covering the PDA medium after the incubation period. Macroscopically, the colonies displayed a circular shape, cottony to powdery texture, and a flat surface, with concentric rings present in most isolates. Obverse coloration ranged from white and yellow to intense green, whereas the reverse showed light to yellowish tones. Microscopic characterization revealed hyaline hyphae, branched conidiophores, ampulliform phialides, and conidia grouped in different configurations (Figure 1), morphological features consistent with *Trichoderma* (Carrasco-López *et al.*, 2024; Castrejón-Noguerón, 2024). However, owing to the phenotypic similarity among species and the morphological plasticity of the genus, taxonomic identification based exclusively on morphological characters may be limited (Surma *et al.*, 2025).

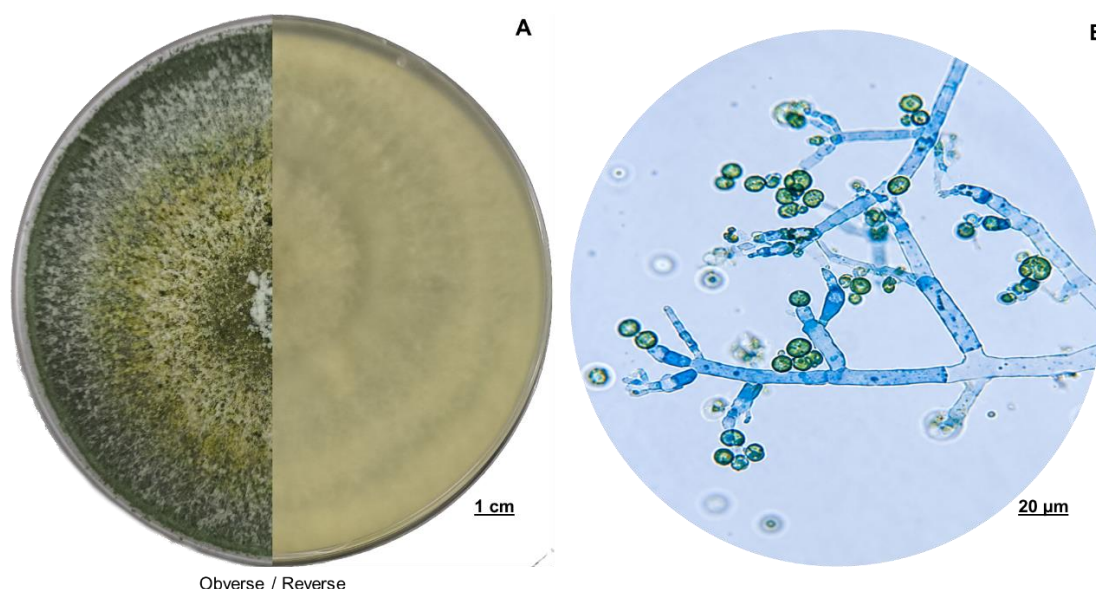


Figure 1. Representative morphological characterization of *Trichoderma*. **A)** Colony morphology observed on potato dextrose agar (PDA), showing the obverse and reverse sides of the colony. **B)** Microscopic structures observed using the slide-culture technique and lactophenol cotton blue staining (100×), showing septate hyphae, branched conidiophores, phialides, and conidia characteristic of the genus *Trichoderma*.

Molecular characterization. Molecular analysis identified *Trichoderma longibrachiatum*, *T. harzianum*, and *T. asperelloides* among the isolates obtained (Table 2). The sequences obtained were deposited in GenBank under accession numbers PZ281653–PZ281660, corresponding to isolates AT1, AT2, AT5, AT6, AT7, AT8, AT9, and TC (Table 2). The phylogenetic tree showed groupings consistent with distinct species of the genus (Figure 2). Isolates AT8 and AT9 clustered with reference sequences of *T. longibrachiatum*, while AT2 was related to *T. asperellum*. The commercial isolate (TC) clustered with *T. asperelloides*. Likewise, AT5, AT6, and AT7 were associated with *T. harzianum*, while AT1 grouped with *Hypocrea lixii*, closely related to *T. harzianum*, forming the *T. harzianum*/*H. lixii* complex. The latter represented the predominant taxon, accounting for four of the seven isolates recovered and present at both sites where the genus was recorded.

Considering the origin of the isolates, representatives of *T. harzianum*/*Hypocrea lixii* and *T. longibrachiatum* were identified in the locality of Palo Blanco, whereas *T. harzianum*/*H. lixii* and *T. asperellum* were identified in the Botanical Garden of the Universidad Autónoma de Guerrero. In terms of species richness, Palo Blanco presented two *Trichoderma* species distributed across five isolates, while the Botanical Garden presented two species represented by two isolates. These results demonstrate the presence of native *Trichoderma* species associated with sites under different management conditions and land use.

Table 2. Sequence length, GenBank accession number, and ITS sequence similarity of *Trichoderma* isolates.

Isolate	Length (bp)	GenBank accession number	Closest matching species	Identity (%)
AT1	595	PZ281653.1	<i>Hypocrea lixii</i>	98
AT2	569	PZ281654.1	<i>Trichoderma asperellum</i>	100
AT5	587	PZ281655.1	<i>Trichoderma harzianum</i>	97
AT6	588	PZ281656.1	<i>Trichoderma harzianum</i>	99
AT7	580	PZ281657.1	<i>Trichoderma harzianum</i>	98
AT8	611	PZ281658.1	<i>Trichoderma longibrachiatum</i>	97
AT9	605	PZ281659.1	<i>Trichoderma longibrachiatum</i>	100
TC	569	PZ281660.1	<i>Trichoderma asperelloides</i>	99

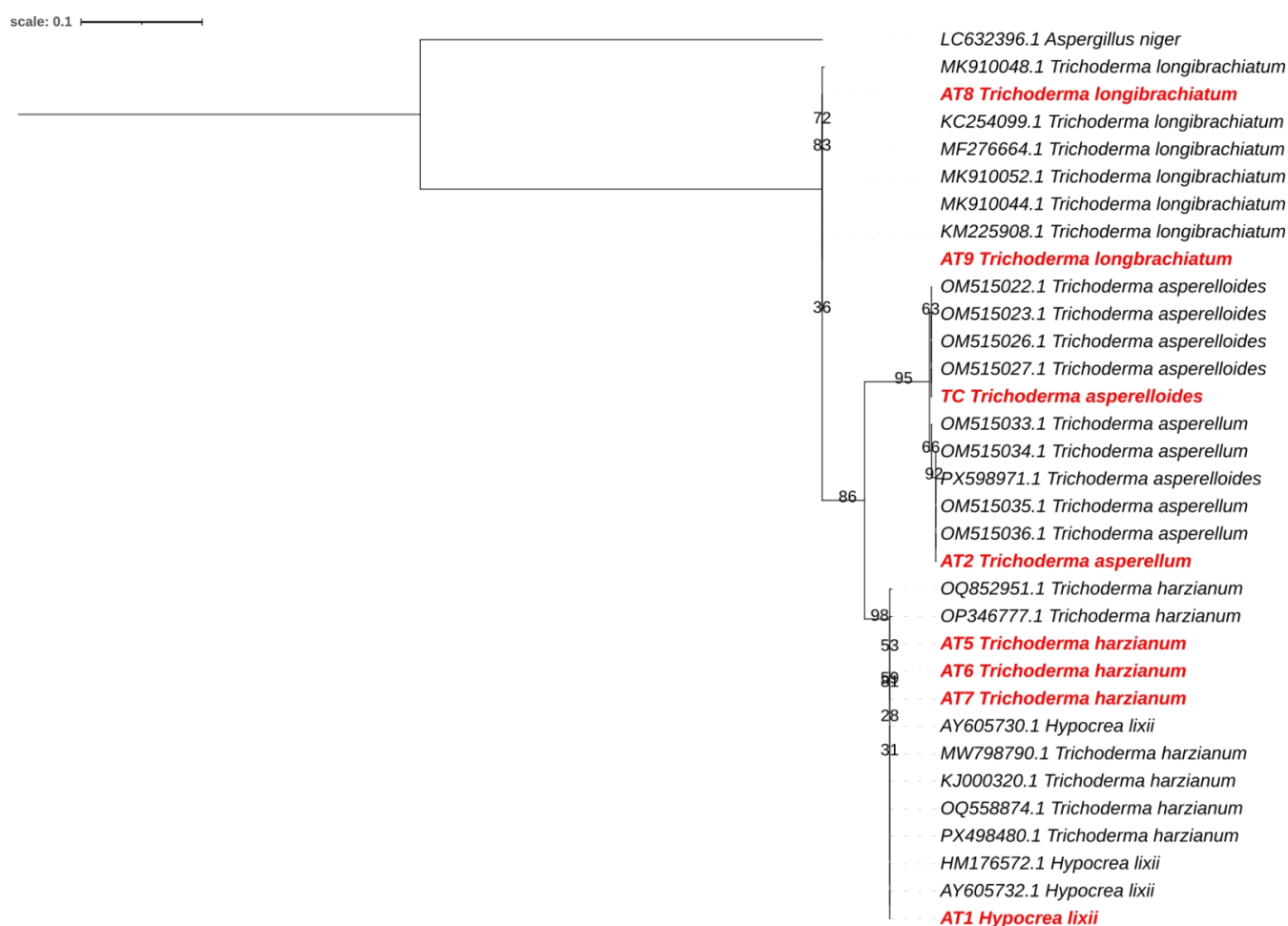


Figure 2. Phylogenetic tree inferred from ITS-region (ITS1–5.8S–ITS2) sequences of *Trichoderma* isolates obtained in this study and reference sequences retrieved from GenBank. The analysis grouped the isolates within the clades corresponding to *T. longibrachiatum*, *T. asperelloides*, *T. asperellum*, *T. harzianum*, and *Hypocrea lixii*. *Aspergillus niger* was used as the outgroup because of its phylogenetic position outside the genus *Trichoderma*, allowing the tree to be rooted and the evolutionary relationships among the analyzed isolates to be interpreted. Node values indicate bootstrap support (%) based on 1,000 replicates.

The species identified are consistent with previous reports from Mexico, where *T. harzianum*, *T. asperellum*, and *T. longibrachiatum* have been recorded in various soil types and agroecosystems and are considered relevant species owing to their frequent association with agricultural environments (Guigón-López *et al.*, 2010; Romero-Rosales *et al.*, 2021; Sánchez-Miranda *et al.*, 2021; Ahedo-Quero *et al.*, 2024). In Guerrero, previous studies have documented similar species associated with roselle (*Hibiscus sabdariffa*) crops and other agricultural systems (Romero-Rosales *et al.*, 2021), suggesting that these taxa form part of the regional soil microbiota. Several studies have highlighted the biotechnological potential of these species as biological control agents and plant growth promoters, owing to their antagonistic capacity against phytopathogens and their production of metabolites of agricultural interest (Poveda, 2021; Afrouz *et al.*, 2023; Jambhulkar *et al.*, 2024). In particular, the predominance of the *T. harzianum*/*H. lixii* complex observed in this study is consistent with reports highlighting its broad ecological adaptability and frequent recovery in agricultural environments (Ismail *et al.*, 2024).

In this context, it is worth noting that the recovery of isolates varied among the sites evaluated, with a higher frequency observed in the locality of Palo Blanco, where five of the seven isolates recovered were obtained. This site is characterized by management based on agroecological principles, including crop diversification, compost use, and reduced dependence on chemical inputs. Several authors have noted that practices of this type can contribute to the maintenance of more diverse and functional microbial communities owing to increased organic matter and reduced soil disturbance (Gupta *et al.*, 2022; Kinsbergen *et al.*, 2025). Although the results obtained do not allow a direct relationship to be established between management and the presence of *Trichoderma*, the greater recovery of isolates in Palo Blanco is consistent with findings reported for systems in which the conservation of soil biological activity is favored.

CONCLUSIONS

Molecular analysis based on the ITS region, complemented by sequencing and phylogenetic analysis, allowed identification of the isolates obtained in the Botanical Garden of the Universidad Autónoma de Guerrero as *Trichoderma harzianum*/*Hypocrea lixii* and *T. asperellum*, whereas the isolates recovered in the locality of Palo Blanco corresponded to *T. harzianum*/*H. lixii* and *T. longibrachiatum*. The *T. harzianum*/*H. lixii* complex was the predominant taxon, representing 57.1% of the isolates recovered.

The results obtained expand the current knowledge of the diversity of native *Trichoderma* species present at sites with different management conditions and land use in the municipality of Chilpancingo de los Bravo, Guerrero, including agroecological systems and areas of plant conservation. They also provide information on the local distribution of this genus in the region. However, since the present study was limited to the isolation, morphological characterization, and molecular identification of the isolates, the potential biotechnological value of the isolates obtained should be corroborated through subsequent studies of antagonism, metabolite production, and functional evaluation.

Limitations. The present study represents an initial approach to the diversity of *Trichoderma* at three sites with different management conditions and land use in the municipality of Chilpancingo de los Bravo. Among the methodological limitations are the number of sites evaluated, the sampling size, and the exclusive use of the ITS region as a molecular marker, which may present limitations for discriminating closely related species

within the genus. Future studies should incorporate multilocus approaches including markers such as TEF1- α and RPB2 to increase taxonomic resolution.

Conflict of interest. The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this Note.

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