



Diversity of *Trichoderma* species in association with banana and weed roots in southeast Mexico †

[Diversidad de especies de *Trichoderma* en asociación con raíces de banano y malezas en el sureste de México]

Carmela Hernández-Domínguez^{*1}, Martín González-Graillet²,
Betzabel Cruz-Garrido¹, Roberto Carlos Ortega-García¹,
Cesar Adriel Santiago-Moreno¹, Fabiel Vázquez-Cruz¹, Delfino Reyes-López¹
and Gabriela Hernández-Ramírez³

¹Facultad de Ciencias Agrícolas y Pecuarias, Benemérita Universidad Autónoma de Puebla. Av. Universidad s/n, San Juan Acateno, Teziutlán Puebla 73965, México.

Email: *carmela.hernandezd@correo.buap.mx, betzycg@hotmail.com,
betoortg98@gmail.com, adrielstgo03@gmail.com, fabiel.vazquez@correo.buap.mx,
delfino_reyes2001@yahoo.com.mx

²Colegio de posgraduados Campus montecillo, km 36.5, Carretera México-Texcoco, Montecillo 56230, Texcoco Edo. de México, México. E-mail: martyn-graillet@hotmail.com

³Tecnológico Nacional de México /IT Superior de Tierra Blanca. Av. Veracruz s/n esquina Héroes de Puebla, Col. Pemex, Tierra Blanca. C.P. 95180, México. E-mail: gabriela.hernandez@itsb.edu.mx

^{*}Corresponding author

SUMMARY

Background. Cultivated plants and weeds play an important role in interacting with soil microorganisms, which, together with climatic and edaphic conditions, characterize the ecological niches of microorganisms such as *Trichoderma*. **Objective.** It was to determine the abundance and diversity of *Trichoderma* species with endophytic and epiphytic association in the root of five varieties of banana and weed in the states of Oaxaca, Veracruz, and Puebla, Mexico. **Methodology.** For the study, a total of 60 samples of banana root and weeds were collected, and from 3 to 7 samples per plot in which the pH and organic matter were measured. The endophytic and epiphytes fungi root was isolated, and these were characterized morphologically and molecularly. For the diversity analysis, the Shannon-Wiener (H') and Simpson indexes were used, and the frequency of species was calculated. **Results.** A total of 23 isolates were obtained, of which 21 were *Trichoderma longibrachiatum*, one of them *Trichoderma reesei* and other *Trichoderma harzianum*, and 61% of 23 isolates were epiphytes and 39% endophytes, and only the *T. longibrachiatum* specie showed both behaviors. Likewise, *T. longibrachiatum* was isolated in all the banana varieties analyzed as well as in weeds, and it had 100% presence in the enano gigante, macho Papaloapan, and macho chifle varieties, while in banana apple tree and purple varieties were obtained *T. harzianum* and *T. reesei* in 8% and 50% respectively. Of the total isolates, 70% were obtained from bananas and 30% from weeds, and none of them were endophytic in weeds. The highest abundance index AI= 0.1 was to Veracruz, which had pH=7.1 and O.M.=2.8-5.7, however, the diversity index in it was 0, the highest diversity indices were H=0.3, 0.2, and Ds= 0.67 which were obtained in Puebla state with pH= 6.7 and O.M.= 6.0 to 10 very similar to optimum. **Implications.** The cultivation of different varieties in each sampled state limited the comparison of determining results in the study. **Conclusion.** The abundance and diversity of *Trichoderma* species, as well as their endophytic and epiphytic behavior in banana roots, are influenced by factors such as the plant variety, which is colonized, the non-cultivated plant species found in the area, as well as the pH and the amount of organic matter in the soil. These factors generate inter and intraspecific relationships that intervene in the frequency of occurrence of *Trichoderma* species.

Key words: abundance; varieties; behavior; plant; microorganisms.

† Submitted May 9, 2023 – Accepted August 4, 2025. <http://doi.org/10.56369/taes.4943>



Copyright © the authors. Work licensed under a CC-BY 4.0 License. <https://creativecommons.org/licenses/by/4.0/>

ISSN: 1870-0462.

ORCID = C. Hernández-Domínguez: <http://orcid.org/0000-0003-0212-216X>; M. González-Graillet: <http://orcid.org/0000-0002-8521-9073>; F. Vázquez-Cruz: <http://orcid.org/0000-0002-4425-6150>; D. Reyes-López: <http://orcid.org/0000-0002-8360-0567>

RESUMEN

Antecedentes. Las plantas cultivadas y malezas desempeñan un papel importante en la interacción con microorganismos del suelo, los cuales, junto con las condiciones climáticas y edáficas caracterizan a los nichos ecológicos de microorganismos como *Trichoderma*. **Objetivo.** Determinar la abundancia y diversidad de especies de *Trichoderma* con asociación endófito y epífita en raíces de cinco variedades de banano y maleza en los estados de Oaxaca, Veracruz y Puebla, México. **Metodología.** Para realizar el estudio, se colectaron 60 muestras de raíz de banano y maleza y se extrajeron de 3 a 7 muestras de suelo a una profundidad de 30 cm por parcela en la cual se midió el pH y la materia orgánica. Se caracterizaron morfológicamente y molecularmente los aislados de hongos endófitos y epífitos obtenidos de raíz. Para el análisis de diversidad se usó el índice de Shannon-Wiener (H'), y el de Simpson y se calculó la frecuencia de especies. **Resultados.** Se obtuvieron 23 aislados, de los cuales 21 fueron de *Trichoderma longibrachiatum*, uno de *Trichoderma reesei* y uno de *Trichoderma harzianum*. El 61% de aislados fueron epífitos y 39% endófitos, y de las tres especies obtenidas, solo *T. longibrachiatum* mostró ambos comportamientos, además de que se aisló en todas las variedades de banano analizadas, así como en maleza, y obtuvo 100% de presencia en las variedades enano gigante, macho Papaloapan y macho chifle, mientras que en manzano y morado se obtuvo *T. harzianum* y *T. reesei* en 8% y 50% respectivamente. Del total de los aislados, 70% fueron obtenidos de banano y 30% de maleza, y ninguno de ellos fue endófito en malezas. El mayor índice de abundancia $IA = 0.1$ fue en Veracruz con pH de suelo 7.1 y M.O 2.8-5.7; sin embargo, el índice de diversidad en ese mismo estado fue 0 y los mayores índices de diversidad fueron $H = 0.3$, 0.2 y $D_s = 0.67$ obtenidos en el estado de Puebla con pH 6.7 y M.O de 6.0 a 10 muy cercanos al óptimo. **Implicaciones.** El cultivo de diferentes variedades de plátano en cada estado muestreado, limitó la comparación de resultados determinantes en el estudio. **Conclusión.** La abundancia y diversidad de especies de *Trichoderma*, así como su comportamiento endófito y epífita en raíces de banano se ven influenciadas por factores como la variedad a la que colonizan, malezas encontradas en el área, así como pH y cantidad de materia orgánica del suelo, ya que dichos factores generan relaciones inter e intraespecíficas que intervienen en la frecuencia de ocurrencia de las especies de *Trichoderma*.

Palabras clave: abundancia; variedades; comportamiento; planta; microorganismos.

INTRODUCTION

Southeastern Mexico is characterized by a wide variety of tropical plant species adapted to the climate and soil of the region (Martínez-Adriano *et al.*, 2016; Martínez-Bernal *et al.*, 2021; Pérez García *et al.*, 2012). Likewise, several crops such as bananas are produced, these crops generate numerous jobs and are a source of foreign exchange (FAOSTAT, 2020). Different varieties can be found in this crop, which have adapted to different types of soils, in the region, in which there is a diversity of microorganisms (Hassani *et al.*, 2018; Kaushal *et al.*, 2022; Nunes *et al.*, 2009) these microorganisms include species of plant pathogenic and beneficial fungi such as *Trichoderma* genus (Candra *et al.*, 2022; Kaushal *et al.*, 2022) that interact with roots, playing an important role (Malgioglio *et al.*, 2022). For example, *Trichoderma viride* improves the capacity of stress tolerance of the plant and establishes a positive interaction; *Trichoderma asperellum* enhances growth, confers pathogens tolerance and produces specialized plant metabolites, in addition to inducing systemic resistance (Castro-Restrepo *et al.*, 2022; Gundrathi and Babu, 2019). So far, several studies have been published about the benefits of *Trichoderma* genus in association with plants, however, there is a lack of research directed towards the endophytic behavior of this fungus with cultivated and uncultivated plants. It is called endophytism when

a microorganism lives asymptotically within the tissue of a host (Hardoim *et al.*, 2015), making it difficult to detect its presence, and even more so, if this is not culturable in a synthetic medium. Endophytic microorganisms establish symbiotic and mutualistic interactions in which plants benefit by providing them with accommodation (Dastogeer *et al.*, 2020). Likewise, it is known that the interaction of endophytic fungi varies depending on the plant or organ and species they colonize (Dastogeer *et al.*, 2017). They can be found in different geographical sites, fulfilling different functions and associated with different types of plants including weeds. For this reason, we investigated the diversity of endophytes and epiphytes associated *Trichoderma* species with roots from five banana varieties and the existing weeds in different plots in the states of Veracruz, Oaxaca, and Puebla in Mexico.

MATERIALS AND METHODS

Sampling sites location

For the present research, 30 root samples were taken at sites in the ejidos of Pueblo Nuevo, Santa Teresa, and San Rafael, in Oaxaca state. In addition, 30 samples were taken in El Pital, Veracruz state, and 30 in Zompanico, Hueytamalco, Puebla state, from February to May 2021 (Table 1).

Table 1. Soil and root sample collection sites of 5 banana and their weeds varieties in Mexico.

Source location	Georeference	Isolated Key	Type of plant	GeneBank
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OI5MO ₂	Banana	OP157537
Zompanico, Puebla	20.00°72'50''-97.34°85'00''	PI5R- O	Banana	OP157538
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIV4M-O	Broadleaf	OP161748
Santa Teresa, Oaxaca	18.08°89'00''-96.11°14'11''	OII4.2	Banana	OP157540
San Rafael, Oaxaca	18.80°19'10''-96.60°12'20''	OIIIM-3	Broadleaf	OP161745
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VII4M-O	Broadleaf	OP161746
Zompanico, Puebla	20.00°72'50''-97.34°85'00''	PIII5R O	Banana	OP161747
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OI3.3	Broadleaf	OP157543
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OII1R-3	Banana	OP157544
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OI5M-3	Banana	OP157545
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OI5RO	Banana	OP157546
Pueblo Nuevo, Oaxaca	18.90°14'00''-96.60°09'90''	OI5MO	Grassy weeds	OP157547
Santa Teresa, Oaxaca	18.08°89'00''-96.11°14'11''	OII1R-3	Banana	OP157548
San Rafael, Oaxaca	18.80°19'10''-96.60°12'20''	OIII3M-3	Grassy weeds	OP157549
San Rafael, Oaxaca	18.80°19'10''-96.60°12'20''	OIII4R-3	Banana	OP157550
Zompanico, Puebla	20.00°72'50''-97.34°85'00''	PIV5RO	Grassy weeds	OP157551
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VI5.1	Banana	OP157552
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VII.2.1	Banana	OP157553
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIII.3	Banana	OP157554
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIV.2.1	Banana	OP157555
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIV.2.3	Banana	OP157556
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIV.3.2	Banana	OP157557
El Pital, Veracruz	20.15°46'65''-96.89°41'02''	VIV.5.1	Banana	OP157558

Sampling of plant and soil material

In an area of 5000 square meter, 3 to 7 samples of 10 g were taken from plant roots banana and weeds, respectively. For each sampled banana, a root sample was taken from a weed found 2 m away. Sampling was carried out using an alcohol-disinfected shovel, through a hole at the base of the plant, its main roots were removed along with 200 g of soil, wrapped in sterile paper towels, and placed in 10 x 10 cm zipper bags (ziploc®). These were labeled with the date, GPS coordinates, locality, and sample type (weed or banana variety). Samples were placed in expanded polystyrene coolers with refrigerants and then transported to the laboratory of the Faculty of Agricultural and Livestock Sciences Benemerita Universidad Autónoma de Puebla for analysis.

Isolation of *Trichoderma* species

Isolation of epiphytic species of *Trichoderma*

A 100 mL of sterile solution with tween 80 at 0.05% was added to each bag with root sample, then the sample was shaken for 1 min or until the soil was detached from the surface of each root. From this solution, a 1:10 dilution was prepared (1 mL of the soil solution with 9 mL solution of sterile Tween 80 at 0.05%) as well as two additional dilutions. Subsequently, two replicates of 100 µL were spread by using a Drigalsky spatula on the surface of the SDA

(Sabouraud Dextrose Agar) culture medium, according to the plate spreading technique methodology described by Goettel and Douglas (1996). The plates were then incubated for 72 h at 28 °C and growth of fungal colonies with characteristics of the *Trichoderma* genus present per replicate was counted. Counted colonies were isolated and reproduced on sterile filter paper and placed on Petri dishes in an SDA culture medium.

Isolation of endophytic species of *Trichoderma*

To isolate endophytic fungi, longitudinal pieces of roots 0.5 to 1 mm thick were washed and cut, and slides added with blue lactophenol according to Subhashini et al. (2016) for observation and detection of endophytic fungi under compound microscopy. Subsequently, samples with endophytic fungi were cut into square pieces of approximately 0.3 x 0.5 cm, washed with 1% sodium hypochlorite for 1 min, rinsed with sterile water, dried with sterile towels, and placed on SDA-containing culture plates under sterile conditions according to the methodology of Agrios (2004). Plates were incubated at 28 °C for 72 h and thereafter, the external growth of root pieces was observed, then isolated and cultured in an SDA medium for morphological identification.

Morphological and molecular characterization of *Trichoderma* species

Morphological

Characterization of colonies was realized through morphologic analysis and measurement of mycelium growth in a PDA culture medium. In addition, observations were made in composite microscopy placing small pieces of fungi on a slide and then covered with glass. The form and type of mycelium, as well as the form and type of spores, were described according to the taxonomic keys reported by Barnett and Hunter (1998).

Molecular

Molecular characterization was carried out in LADIFIT S. A de C.V. facilities located at Colegio de Postgraduados Campus Montecillos. For the molecular characterization, ITS4, ITS5, ITS1 primers from internal transcription spacer (ITS, 5.8s rDNA gene) and TEF1 and TEF2 primer from EF1-alpha gene were used, according to reliable extraction and amplification protocols.

Statistical analysis

For the analysis of *Trichoderma* species diversity, they were used the Shannon-Wiener (H') and Simpson indexes. Species frequency (F) was analyzed according to Ayoub *et al.* (2020); Mo *et al.* (2006), and Magurran (2013). The Shannon index (H') was determined with the formula: $H' = -\sum_{i=1}^n \frac{X_i}{N} \ln \frac{X_i}{N}$. Where (H') is the Shannon-Wiener index, X_i is the number of observations of species " i ", N is the total number of isolates observed in each sample, \ln is the assigned logarithm which is at least 0, and n is the number of individuals or species. Simpson's Index was calculated using the following formula: $D_s = 1 - \frac{\sum n_i(n_i-1)}{N(N-1)}$. Where (D_s) means Simpson's Index, S = Number of species, N = Total of organisms, N = number of specimens per species. Occurrence frequencies (F) for each species were calculated as follows: $F = ((\text{number of individuals of one species}) / (\text{number of individuals of all species})) * 100$

Frequency measures represent the occurrence of a phenomenon in populations and therefore, are fundamental for descriptive and analytical research, as they permit describing an event about the population size.

RESULTS AND DISCUSSION

Morphological and Molecular Characterization

Morphology of the isolates of *Trichoderma* Pers. (Hypocreales, Hypocreaceae) genus observed in this study, showed the existence of three species: *T. longibrachiatum*, *T. reseei*, and *T. harzianum*. *T. longibrachiatum* was characterized by sparse aerial mycelium (Figure 2A) with small cottony pustules (Figure 2B), typically ellipsoid to oblong, smooth, subglobose or rough to tuberculate conidia (Figure 2C). Well-developed conidiophores (Figure 2D) from which individual or whorled phialides arise (Figure 2D and 2E), very frequently, a single phialide terminating with a spur-like basal cell (Figure 2F), phialides are commonly cylindrical and often sinuous. These results agree with those described by Montoya *et al.* (2016).

The characteristics observed for *T. reseei* are coincident with those described by Atanasova *et al.* (2010) who reported *T. reseei* (teleomorph *Hypocrea jecorina*; Hypocreales, Ascomycota, Dikarya) with the presence of mycelium covering the Petry dish after 3 or 4 days at 25 to 37 °C with emission of conidia after 4 to 8 days at 25 °C, grouped in a distal concentric zone, farinose, green tending to yellow (Figure 3A). Conidia with non-very well-defined pustules forming bush-like colonies, variable, ellipsoidal, or oblong with parallel sides (Figure 3B y C), Chlamydospores are small, oblong, and often form separate chains (Figure 3D), Conidiophores are straight, with 2.5 to 5 microns wide, bush-like, larger branches becoming thick with warts over time (Figure 3E y 3F). Phialides are solitary, lageniform or ampuliform, straight and rarely slightly curved, sometimes with long cylindrical necks (Figure 3G).

On the other hand, *T. harzianum* exhibited white mycelium that changed to light green after 4 days with darker-colored annular zones (Figure 4A). In colonies with smooth surfaces and white aerial mycelium, growth was 4 to 5 days (Figure 4B). Conidia subglobose or short ovoid, often with a truncated base, and perfectly smooth walls (Figure 4C). Loose, highly branched conidiophores forming tufts (Figure 4D). Phialides with short bolus anatomy, bulging in the middle and narrower at the base arising singly (Figure 4E), Chlamydospores slightly elongated (Figura 4G). The description observed for *T. harzianum* is like that reported by Kumar *et al.* (2019).

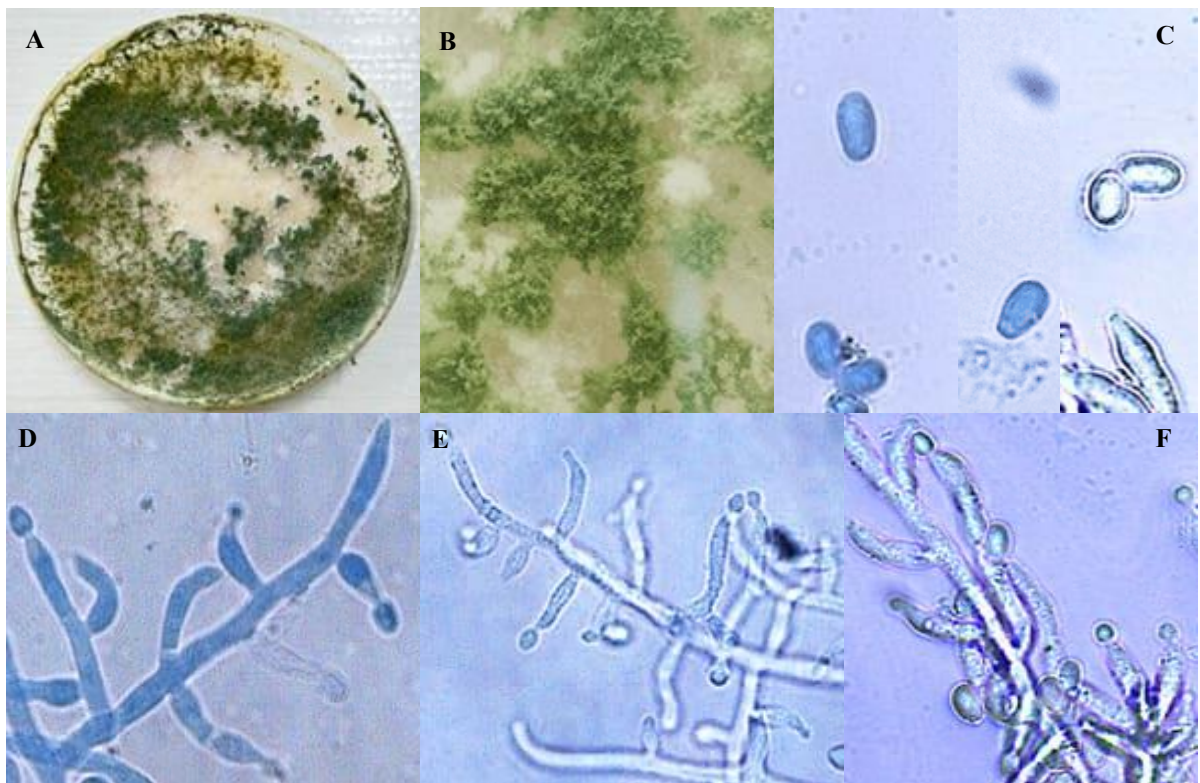
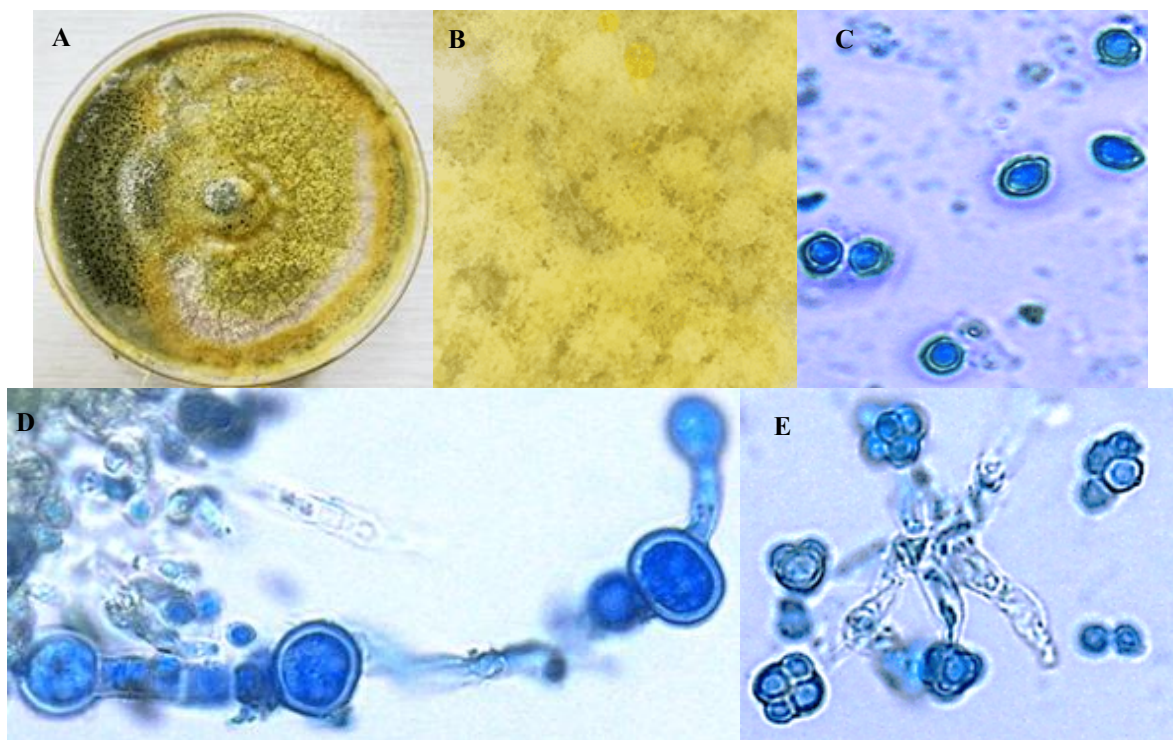


Figure 2. Morphological characteristics of *T. longibrachiatum*, isolate OIII3M -3 after 8 days at 30 °C cultured on SDA (A), mycelial growth observed at 40X forming pustules on SDA culture medium (B), oblong conidia observed at 100X (Figure C), Individual phialides (D and E), Spur-terminated phialides (F).



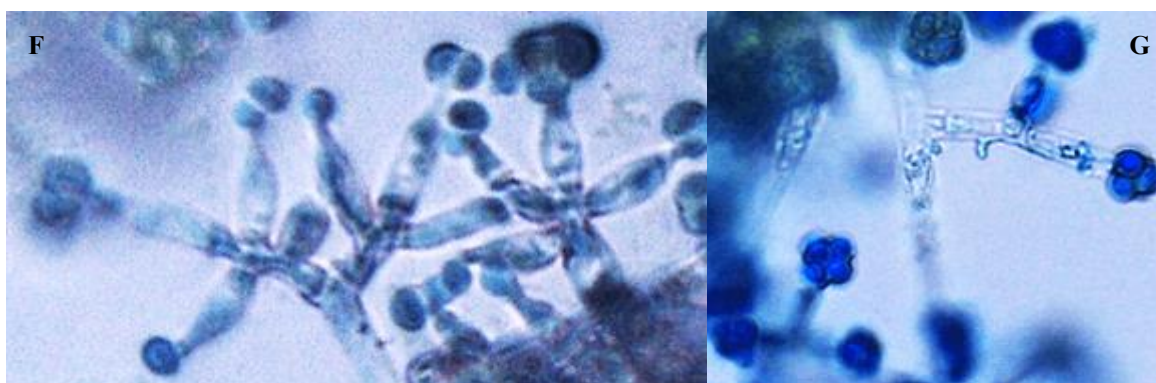


Figure 3. Morphological characteristics of *T. reesei* A-G. Isolate OI5MO₂ after 8 days at 30 °C cultured on Sabouraud Dextrose Agar (A), mycelial growth observed at 40X with amorphous pustules on SDA culture medium (B), ellipsoidal or oblong conidia with parallel sides (C), Chlamydospores form a chain (D), bushy phialides observed at 100X (E y F), individual phialides. (G)

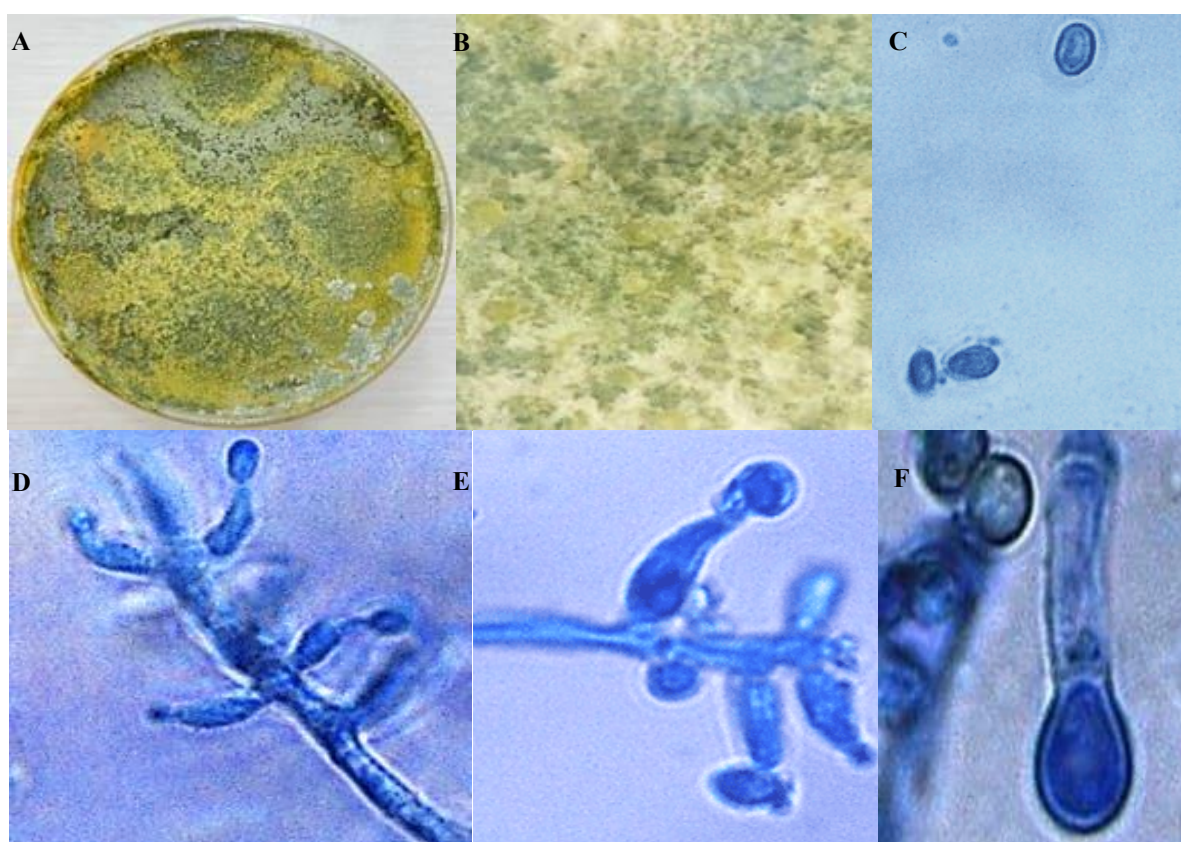


Figure 4. A-F *T. harzianum* isolate OI5MO₂ (A). Light green mycelium after 4 days on SDA medium at 30 °C colonies with almost smooth surfaces and aerial mycelium (B). Subglobose conidia with truncated base (C). Loose conidiophores (D). Bulging phialides in the middle and narrower at the base (E). Chlamydospores slightly elongated (F).

A total of 23 DNA sequences of *Trichoderma*: isolates were obtained in this study and according to the sequence comparison using the GeneBank (NCBI) tools, 21 sequences were aligned to corresponding

sequences of the species *T. longibrachiatum*, one to *T. reesei* and one to *T. harzianum* (Figure 5), the latter already reported in Mexico (Allende et al., 2022).

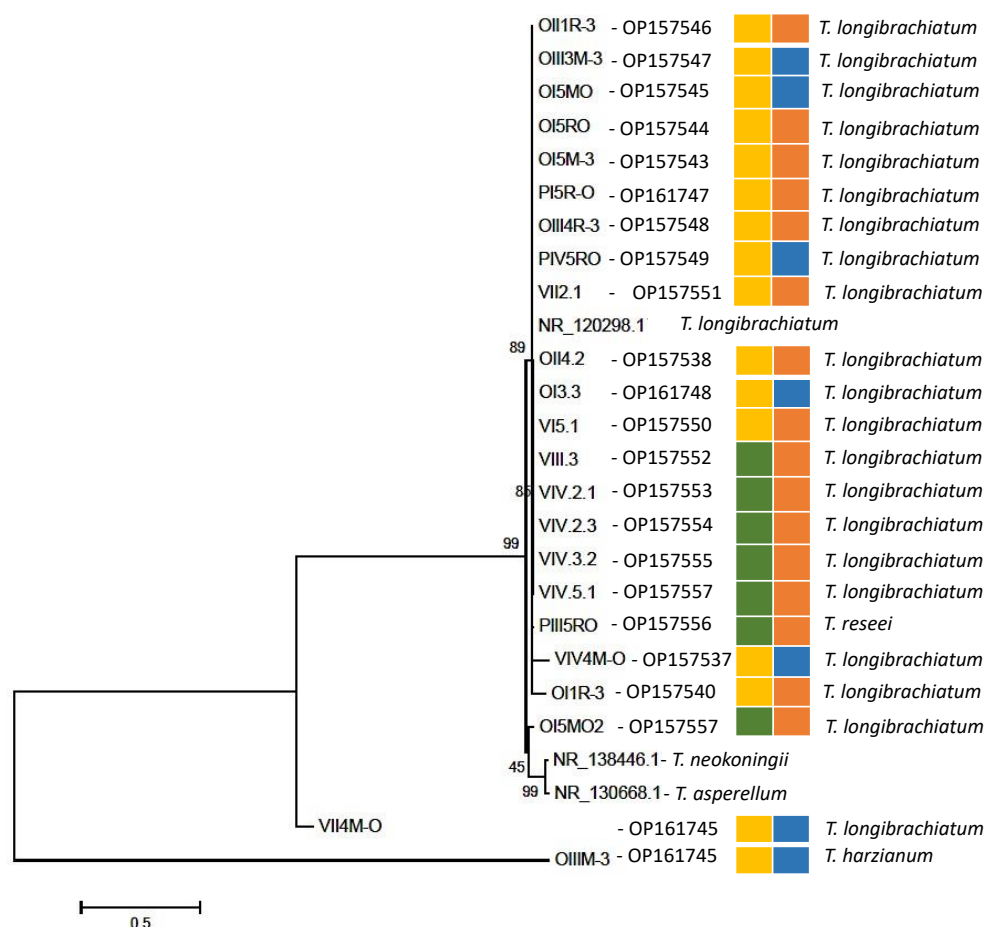


Figure 5. Phylogeny of *Trichoderma* species inferred by Maximum Likelihood (ML), analysis of ITS gene data, 5.8s rDNA. Only bootstrap values above 80% are shown. Endophyte 39% (■), epiphyte 61% (■), banana 70% (■), Weed 30% (■).

Genetic diversity of endophytic and epiphytic isolates of *Trichoderma* in bananas and weeds

The results showed low genetic diversity for endophytic isolates, in contrast with authors such as Atanasova (2014); Plessis *et al.* (2018) who mention the cosmopolitan and diverse distribution of this genus in different ecological niches. Of the 23 isolates obtained, 13 were epiphytes and 10 endophytes, representing 61% and 39% respectively (Figure 5), it was observed that most of the isolates obtained were epiphytes. In this regard, Tseng *et al.* (2020) inoculating *Arabidopsis thaliana* and *Nicotiana attenuata* plants with *Trichoderma confertum*, *Trichoderma pleuroticola*, *Trichoderma pleuroti* and *T. longibrachiatum* epiphytic colonization after two days and endophytic colonization after 7 days were

observed in the later development stages and less difference between the number of non-colonized and endophytically colonized plants, which suggests that there is endophytic colonization at different stages of plant development, as indicated by Gómez-Lama *et al.* (2021), who mentioned that the phenological stage is determinant in the recruitment and organization of the endophytic microbiome of the plant. Likewise, studies carried out by Ramírez-Torres *et al.* (2022) on two *in vitro* banana varieties showed that the *Trichoderma* species used in the study have an influence on the percentage of endophytism, so it is possible to observe that the isolates obtained, only the species *T. longibrachiatum* showed endophytic behavior as well as epiphytic, while *T. reesei* and *T. harzianum* presented epiphytic behavior (Figure 6).

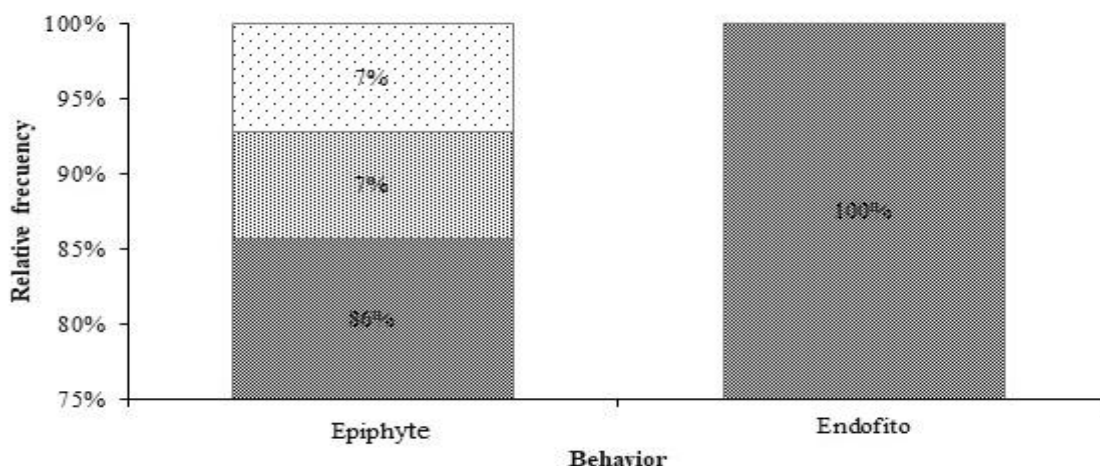


Figure 6. The behavior of three species of *Trichoderma* isolated from banana and weed: *T. harzianum* (□), *T. reesei* (▤), *T. longibrachiatum* (▨).

On the other hand, of the total of *Trichoderma* isolates, 70% were from bananas and 30% from weeds (Figure 5), which showed the preference of the genus to coexist with cultivated plants. Similar observations were reported by Zhu *et al.* (2020) who found tolerance of *Triticum aestivum*, *Hordeum vulgare*, *Vicia faba*, and *Pisum sativum* to *Trichoderma polysporum* (Louk:Fr.) Rifai, while in the weeds *Elsholtzia densa* Benth, *Avena fatua* L, and *Polygonum lapathifolium* L., this same species caused mortality. There are few studies showing greater coexistence of *Trichoderma* with cultivated plants than with weeds. However, it has been observed in banana production plots that the existence of weeds is determinant, as shown by Hernández-Domínguez *et al.* (2019), who reported 65% more CFU of *Fusarium* and *Trichoderma* per gram of soil without weed removal, compared to soil without weeds, suggesting that interaction of cultivated plants and weeds influences the number of species found.

Likewise, no *Trichoderma* endophyte species were obtained in weeds, suggesting little interaction of the genus with this type of behavior. In this regard, studies by Mukhtar *et al.* (2010) showed that the communities of endophytic fungi in four species of weeds were remarkably few in contrast to the communities of epiphytes, while *Trichoderma* was only found in the epiphytic form. On the other hand, Catambacan *et al.* (2021) isolated *Trichoderma asperellum* from the weed *Commelina diffusa* which grew in plots planted with Cavendish banana, which demonstrated the existence of some endophyte species in weeds, whose interaction depends on the existing *Trichoderma* species.

Abundance and diversity of endophytic and epiphytic isolates of *Trichoderma* in banana varieties in the states of Puebla, Veracruz, and Oaxaca

The present results showed that *T. longibrachiatum* was identified in all the varieties of banana analyzed, as well as in weeds, where it had 100% presence in the enano gigante, macho Papaloapan, and macho chifle varieties, while in the manzano and morado varieties they obtained other *Trichoderma* species such as *T. harzianum* and *T. reesei* in 8% and 50% respectively (Figure 7).

In this regard, Berg *et al.* (2005) observed that *Trichoderma* species showed a high degree of specificity in plants, however, several studies have shown that the consequence of colonization of plants varies depending on the growth, presence of environmental factors, soil nutrients, and soil conditions (Contreras-Cornejo *et al.*, 2016), likewise, the type of plant plays an important role in the presence of fungi, and the exudates of its roots cause that some *Trichoderma* species are more attracted to them (Okoth *et al.*, 2007).

On the other hand, from fungal isolates obtained in the municipalities of San Rafael, Santa Teresa, and Pueblo Nuevo Oaxaca, the species *T. longibrachiatum* was obtained more frequently in 91%, while *T. harzianum* was only present in 9%. For Puebla state, *T. longibrachiatum* represented 67%, and *T. reesei* 33% of the isolates found in the municipality of Huehuetamalco (Zompanico village). In San Rafael municipality, state of Veracruz, 100% of the species *T. longibrachiatum* was obtained (Figure 7). It was observed that the highest abundance index was for *T. longibrachiatum* = 0.1 in Veracruz state and the lowest index was for *T. harzianum* = 0.090 in Oaxaca state (Table 2).

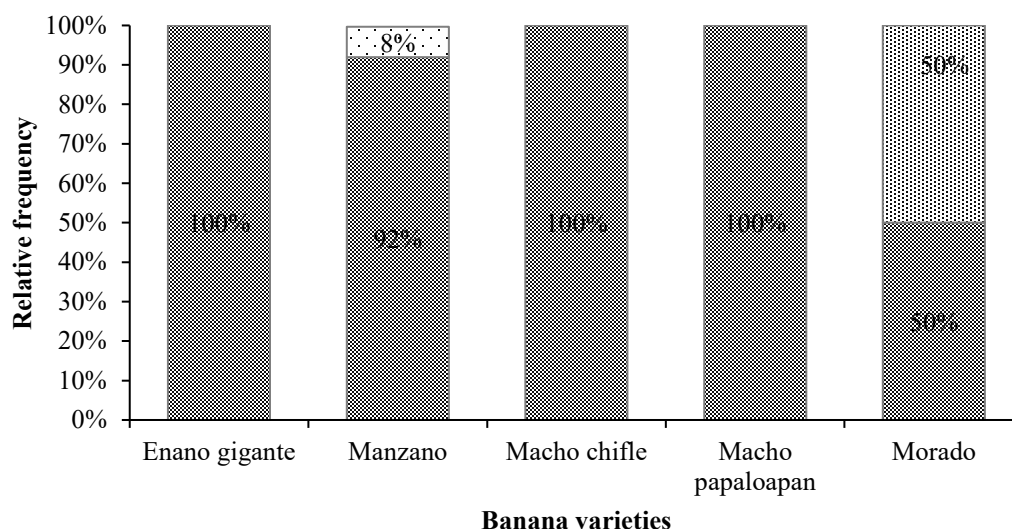


Figure 7. Frequency of *Trichoderma* species obtained in five banana varieties. *T. harzianum* (□), *T. reesei* (▨), *T. longibrachiatum* (▩).

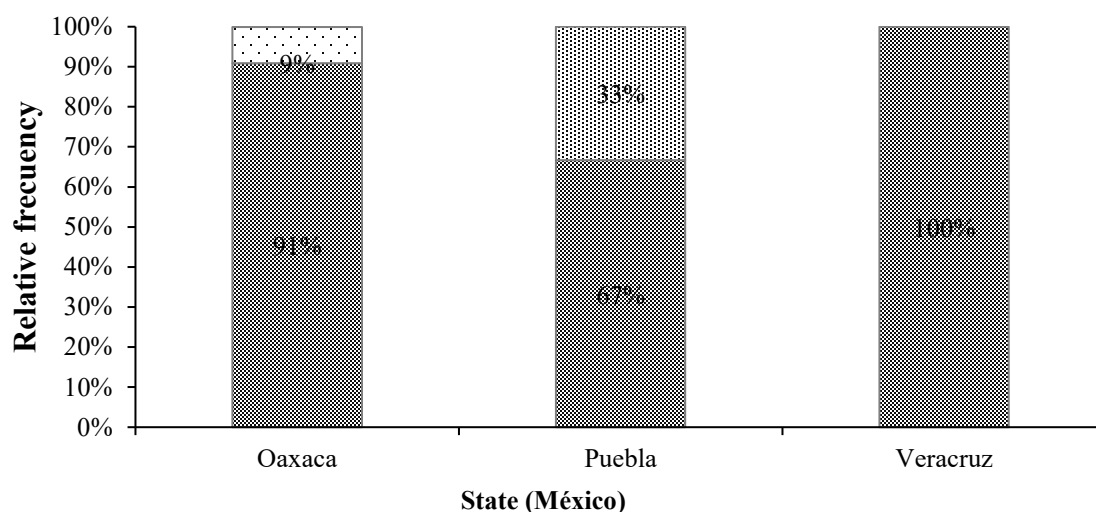


Figure 8. Frequency of *Trichoderma* species obtained from different Mexican states. *T. harzianum* (□), *T. reesei* (▨), *T. longibrachiatum* (▩).

Table 2. Species information of different banana varieties and soil properties.

State	Species	AI of species $P_i = n/N$	$H = -H = -\sum p_i (\log p_i)$	$D_s = 1 - \frac{\sum n_i(n_i-1)}{N(N-1)}$	pH	O. M.
Oaxaca	<i>T. harzianum</i>	0.090	0.216	0.1818	7.3	3.0-5.2 too low
	<i>T. longibrachiatum</i>	0.90	0.094			
Puebla	<i>T. reesei</i>	0.66	0.366	0.67	6.7	6.0-10.0 Low-medium
	<i>T. longibrachiatum</i>	0.33	0.270			
Veracruz	<i>T. longibrachiatum</i>	0.1	0	0	7.1	2.8-5.7 too low

AI=Abundance index, H=Shannon's diversity index, D_s=Simpson's diversity index, O.M: organic matter.

There are several factors involved in the abundance of *Trichoderma* species in soil, however, the physicochemical conditions of the rhizosphere influence microbes proliferation (Husson, 2013; Pelagio-Flores *et al.*, 2017), where pH is one of the most important parameters for *Trichoderma* spp. growth since they grow better in acidic conditions with pH between 4 and 6 (Brady and Weil, 1999; Pelagio-Flores *et al.*, 2017). However, soils from Veracruz state with pH 7.1 and O.M. 2.8-5.7 showed an abundance index AI= 0.1, its diversity index was 0 which shows that some species such as *T. longibrachiatum* are able to adapt to soils with pH higher than 6.0, however, the low amount of organic matter can limit the diversity of *Trichoderma* species (Marín-Guirao *et al.*, 2015; Naumova *et al.*, 2022). It was also observed that the highest diversity indexes were H=0.3, 0.2, and Ds= 0.67 obtained in soils from the Puebla state with pH 6.7 and O.M. from 6.0 to 10 very close to the optimum (Table 2), which agrees with Naumova *et al.* (2022); Pelagio-Flores *et al.* (2017), who mentioned the adaptation of most species to these conditions.

CONCLUSION

The abundance and diversity of *Trichoderma* species, as well as its endophytic and epiphytic behavior in banana roots, are influenced by the variety of plants they colonize, weed species found in the area of influence, as well as soil characteristics, such as pH and amount of organic matter. However, conditions generated among the interaction of plants and microorganisms, regardless of their status, beneficial or parasite-pathogenic, make the ecological niches unique and irreplaceable in nature, since inter- and intraspecific relationships are generated that characterize the interaction, with some of them showing greater specificity that can be measured as the frequency of occurrence in the sampled sites.

Acknowledgments

The authors would like to thank the Benemérita Universidad Autónoma de Puebla for the use of its facilities and equipment used for the development of this research.

Funding. All authors declare not having received any funding in the development of our research.

Conflict of interest. The authors declare that they have no competing interests.

Compliance with ethical standards Not applicable to this study.

Data availability. Data are accessible through the corresponding author Carmela Hernández Domínguez, carmela.hernandezd@correo.buap.mx.

Author contribution statement (CRediT). **C. Hernández-Domínguez** – Conceptualization, data collection, visualization, writing, review and editing. **M. González-Graillet** – Data collection. **B. Cruz-Garrido** – Conceptualization, writing, review and editing. **R.C. Ortega-García** – Conceptualization, data collection. **C.A. Santiago-Moreno** – Data collection. **F. Vázquez Cruz** – Formal analysis. **D. Reyes López** – Writing - review and editing. **G. Hernández Ramírez** – Writing - review and editing.

REFERENCES

- Agrios, G., 2004. Fitopatología; 4ª edición Ciudad de México, Edit. Limusa pp. 3-741.
- Allende-Molar, R., Báez-Parra, K.M., Salazar-Villa, E. and Rojo-Báez, I., 2022. Biodiversity of *Trichoderma* spp. in Mexico and its potential use in agriculture. *Tropical and Subtropical Agroecosystems*, 25 (3), pp. 088. <https://doi.org/10.56369/tsaes.4297>
- Atanasova, L., Jaklitsch, W.M., Komoń-Zelazowska, M., Kubicek, C.P. and Druzhinina, S.I., 2010. Clonal Species *Trichoderma parareesei* sp. nov. Likely Resembles the Ancestor of the Cellulase Producer *Hypocrea jecorina*/T. reesei. *Applied and Environmental Microbiology*, 76(21), pp. 7259–7267. <https://doi.org/10.1128/AEM.01184-10>
- Atanasova, L., 2014. Ecophysiology of *Trichoderma* in genomic perspective. In: Gupta V, Schmoll M, Herrera- Estrella, A.R., Upadhyay, Druzhinina, I. S., Tuohy M, eds. Biotechnology and biology of *Trichoderma*. Amsterdam, The Netherlands. Elsevier, pp. 25–40. <https://doi.org/10.1016/B978-0-444-59576-8.00002-3>
- Barnett, H. L. and Hunter, B.B., 1998. Illustrated Genera of Imperfect Fungi. London, EUA: Amer Phytopathological Society. pp. 1-218.
- Candra, R.T., Prasasty, V.D. and Karmawan, L.U., 2022. Biochemical Analysis of Banana Plants in Interaction between Endophytic Bacteria *Kocuria rhizophila* and the Fungal Pathogen *Fusarium oxysporum* f. sp. *cubense* Tropical Race (Foc TR4). *Biology and Life Sciences Forum*, 11(84), pp. 2-8. <https://doi.org/10.3390/IECPS2021-11990>
- Catambacan, D.G. and Cumagun, C.J.R., 2021. Weed-Associated Fungal Endophytes as Biocontrol Agents of *Fusarium oxysporum* f. sp. *cubense* TR4 in Cavendish Banana. *Journal of Fungi*,

- 7(3), p. 224.
<https://doi.org/10.3390/jof7030224>
- Dastogeer, K.M.G., Li, H., Sivasithamparam, K., Jones, M.G.K. and Wylie, S.J., 2017. *Host Specificity of Endophytic Mycobiota of Wild Nicotiana Plants from Arid Regions of Northern Australia. Microbial Ecology*, 75(1), pp. 74–87. <https://doi.org/10.1007/s00248-017-1020-0>
- Du Plessis L.I., Druzhinina, S.I., Atanasova, L., Yarden, O. and Jacobs, K., 2018 The diversity of *Trichoderma* species from soil in South Africa, with five new additions, *Mycology*, 110(3), pp. 559-583, DOI: [10.1080/00275514.2018.1463059](https://doi.org/10.1080/00275514.2018.1463059)
- Faeth, S. and Bultman, T., 2002. Endophytic fungi and interactions among host plants, herbivores, and natural enemies. In T. Tscharntke and B. Hawkins (Eds.), *Multitrophic Level Interactions*. Cambridge: Cambridge University Press. pp. 89-123
<https://doi.org/10.1017/CBO9780511542190>
- Goettel, M.S and Douglas, G.I., 1996. Fungi: Hyphomycetes. In. *Manual of Techniques in Pathology* (Eds Lacey, L. A) Montpellier, France. pp. 221-223.
<https://doi.org/10.1016/B978-012432555-5/50013-0>
- Gómez-Lama Cabanás, C., Fernández-González, A.J., Cardoni, M., Valverde-Corredor, A., López-Cepero, J., Fernández-López, M. and Mercado-Blanco, J., 2021. The Banana Root Endophytome: Differences between Mother Plants and Suckers and Evaluation of Selected Bacteria to Control *Fusarium oxysporum* f.sp. *cubense*. *Journal of Fungi*, 7(3), pp. 2-26.
<https://doi.org/10.3390/jof7030194>
- Haixia, Z.h.U., Yongqiang, M.A., Qingyun, G. and Bingliang, X.U., 2020. Biological weed control using *Trichoderma polysporum* strain HZ-31, *Crop Protection*. Volume 134, (April), 105161,
<https://doi.org/10.1016/j.cropro.2020.105161>.
- Hallouti, A., Hamza, A.M., Zahidi, A., Hammou, A.R., Bouharroud, R., Aoumar, B.A.A. and Boubaker, H., 2020., Diversity of entomopathogenic fungi associated with Mediterranean fruit fly (*Ceratitidis capitata* (Dipteral: Tephritidae)) in Moroccan Argan forests and nearby area: impact of soil factors on their distribution. *BMC Ecology*, 64(20), pp. 2-13. <https://doi.org/10.1186/s12898-020-00334-2>
- Hardoim, P., Van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M. and Sessitsch, A., 2015. The HiddenWorld within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Review*, 79 (3), 293–320.
- Hassani1, A.M., Duran, P. and Hacquard, S., 2018. Microbial interactions within the plant holobiont. *Microbiome*, 6 (58), pp. 2-17.
<https://doi.org/10.1186/s40168-018-0445-0>
- Hernández Domínguez, C., Vázquez Benito, J.A, Vázquez Moreno, F., Berdeja Arbeu, R., Morales Fernandez, S.D. and Reyes Lopez, D., 2019. Abundancia y Diversidad genética De *Fusarium Oxysporum* y *Trichoderma* sp., en Musa AAB. *Revista Mexicana De Ciencias Agrícolas*, 10(8), pp. 1783-1796.
<https://doi.org/10.29312/remexca.v10i8.1831>
- Kaushal, M., Swennen, R. and Mahuku, G., 2020. Unlocking the Microbiome Communities of Banana (*Musa* spp.) under Disease Stressed (*Fusarium wilt*) and Non-Stressed Conditions. *Microorganisms*, 8(3), pp. 2-443.
<https://doi.org/10.3390/microorganisms8030443>
- Kumar, V., Verma, K.D., Abhay, K. Pandey, A.K. and Srivastava, S., 2019. *Trichoderma* spp.: Identification and Characterization for Pathogenic Control and its Potential Application. Microbiology for Sustainable Agriculture, Soil Health, and Environmental Protection. New York, Edition 1st Imprint Apple Academic Press, pp. 36-40.
<https://doi.org/10.1201/9781351247061>
- Magurran, A.E., 2013. Measuring biological diversity. New York, Blackwell publishing. pp. 3-132.
https://books.google.com.mx/books/about/Measuring_Biological_Diversity.html?id=fIjsa_xmL_S8C&redir_esc=y
- Malgioglio, G., Rizzo, G.F., Nigro, S., Lefebvre du Prey, V., Herforth-Rahmé, J., Catara, V. and Branca, F., 2022. Plant-Microbe Interaction in Sustainable Agriculture: The Factors That May Influence the Efficacy of PGPM Application. *Sustainability*, 14, (February) 2253. pp. 2-28.
<https://doi.org/10.3390/su14042253>

- Marín-Guirao, J.I., Rodríguez-Romera, P., Lupión-Rodríguez, B., Camacho-Ferre, F. and Tello-Marquina, J.C., 2016. Effect of *Trichoderma* on horticultural seedlings' growth promotion depending on inoculum and substrate type. *Journal Applied Microbiology*, 121(4), pp. 1095-1102.
<https://doi.org/10.1111/jam.13245>
- Martínez-Adriano, C.A., Aguirre-Jaimes, A. and Díaz-Castelazo, C., 2016. Relevamiento florístico de plantas con flores en un ecosistema costero tropical en Veracruz, México. *Ciencias Botánicas*, 94(1), pp. 185-197.
<https://doi.org/10.17129/botsci.272>
- Martínez-Bernal, A., Vásquez-Velasco, B., Ramírez-Arriaga, E., Zárate-Hernández, M. R., Martínez-Hernández, E. and Téllez-Valdés, O., 2021. Composition, structure and diversity of tree and shrub strata in a tropical deciduous forest at Tehuacán Valley, Mexico. *Revista Mexicana de Biodiversidad*, 92 (Noviembre), e923713
<https://doi.org/10.22201/ib.20078706e.2021.92.3713>
- Mo, M.H., Chen, W.M., Su, H.Y., Zhang, K.Q., Duan, C.Q. and He, D.M., 2006. Heavy metal tolerance of nematode-trapping fungi in lead-polluted soils. *Applied Soil Ecology*, 31 (1–2), pp. 11–19.
<https://doi.org/10.1016/j.apsoil.2005.04.008>
- Mohammad Golam Dastogeer, K., Oshita, Y., Yasuda, M., Kanasugi, M., Matsuura, E., Xu, Q. and Okazaki, S., 2020. Host Specificity of Endophytic Fungi from Stem Tissue of Nature Farming Tomato (*Solanum lycopersicum* Mill.) in Japan. *Agronomy*, 10, (July), 1019, pp. 2–17.
<https://doi.org/10.3390/agronomy10071019>
- Montoya, Q.V., Meirelles, L.A., Chaverri, P. and Rodrigues, A., 2016. Unraveling *Trichoderma* species in the attine ant environment: description of three new taxa. *Antonie Van Leeuwenhoek*, 109(5), pp. 633–51. doi:[10.1007/s10482-016-0666-9](https://doi.org/10.1007/s10482-016-0666-9)
- Mukhtar, I., Khokhar, I., Mushtaq, S. and Ali, A., 2010. Diversity of epiphytic and endophytic microorganisms in some dominant weeds. *Pakistan Journal of Weed Science Research*, 16(3), pp. 287–297.
<https://search.ebscohost.com/login.aspx?direct=true&db=edb&AN=67145686&lang=es&site=eds-live>
- Naumova, N., Barsukov, P., Baturina, O., Rusalimova, O. and Kabilov, M., 2022. Soil Mycobiome Diversity under Different Tillage Practices in the South of West Siberia, 12 (July), 1169.
<https://doi.org/10.3390/life12081169>
- Nunes de Jesus, O., Fortes-Ferreira, C., De Oliveira-E Silva, S., Rangel-Camara, T., Leila-Soares, T. and Nogueira-Pestana K., 2009. Characterization of recommended banana cultivars using morphological and molecular descriptors. *Crop Breeding and Applied Biotechnology*, 9(1), pp. 164–173.
- Okoth, A.S., Roimen, H., Mutsotso, B., Muya, E., Kahindi, J., Owino, J.O. and Okoth, P., 2007. Land use systems and distribution of *Trichoderma* species in Embu region, Kenya. *Tropical and Subtropical Agroecosystems*, 7 (2), pp. 105 – 122.
<https://www.redalyc.org/pdf/939/93970205.pdf>
- Pelagio-Flores, R., Esparza-Reynoso, S., Garnica-Vergara, A., López-Bucio, J. and Herrera-Estrella, A., 2017. *Trichoderma*-Induced Acidification Is an Early Trigger for Changes in Arabidopsis Root Growth and Determines Fungal Phytostimulation. *Frontiers Plant Science*, 8 (May), 822.
<https://doi.org/10.3389/fpls.2017.00822>
- Pérez-García, E.A., Meave, J.A. and Cevallos-Ferriz, S.R. S., 2012. Flora and vegetation of the seasonally dry tropics in Mexico: origin and biogeographical implications. *Acta Botánica Mexicana*. 100 (1), pp. 149–193.
https://www.researchgate.net/publication/258498940_Flora_and_vegetation_of_the_seasonally_dry_tropics_of_Mexico_Origin_and_biogeographical_implications
- Robinson, J. and Galán, V., 2011. Plagas: Papel de hongos y bacterias endófitos en el control de las plagas y enfermedades del banano. In: Plátanos y Bananas. Ediciones Mundi-Prensa, España. pp. 265–267.
- Tseng, Y.-H., Rouina, H., Groten, K., Rajani, P., Furch, A.C.U., Reichelt, M., Baldwin, I.T., Nataraja, K.N., Uma Shaanker, R. and Oelmüller, R., 2020. An Endophytic *Trichoderma* Strain Promotes Growth of Its Hosts and Defends Against Pathogen Attack. *Frontiers in Plants Science*. 11(December), 573670.
<https://doi.org/10.3389/fpls.2020.573670>
- Xia, X., Lie, T.K., Quian X., Zheng, Z., Huang, Y. and Shen, Y., 2010. Species Diversity, Distribution, and Genetic Structure of

Endophytic and Epiphytic *Trichoderma* 52
Associated with Banana Roots. *Microbial
Ecology*, 61 (3), pp. 619-625.
<http://doi.org/10.1007/s00248-010-9770-y>