International Journal of Food Microbiology 384 (2023) 109909

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Fungal communities in Brazilian cassava tubers and food products

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ARTICLE INFO

Keywords:
Mycobiota
Cassava root
Tapioca
Starch
Fusarium
Penicillium

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is one of the most widely cultivated foods in the world and is of great socio-economic importance, especially in developing countries. It is predominantly consumed in boiled form, but also is used to produce a number of products, including cassava starch, sour starch, cassava flour and tapioca flour (hydrated cassava starch). Fungal spoilage can occur throughout the production chain, impairing both productivity and quality, as well as posing a potential risk of contamination by mycotoxins. We used multidisciplinary approaches based on phenotypic and molecular data (*ITS/BenA/TEF-1α/RPB2* loci) to investigate the mycobiota of 101 samples (including roots, soil and products) collected in the state of São Paulo, Brazil. A total of 20 fungal groups/genera were morphologically characterized, and 37 different species were molecularly identified. The predominant groups in cassava tubers were *Fusarium* spp., *Penicillium* spp. and *Trichoderma* spp. In cassava products, the most frequent groups were *Penicillium* spp. and *Paecilomyces* spp. Potentially toxigenic species were also found, including *Paecilomyces saturatus, Penicillium citrinum, P. paneum, P. brevicompactum, P. chrysogenum*, *Fusarium foetens* and *Fusarium mundagurra*. In soil-cultivated cassava samples, the groups found most frequently were *Penicillium* spp., *Cladosporium* spp. and *Fusarium* spp. Some of the species found in cassava tubers and/or product samples were also present in the soil, including *F. mundagurra*, *Neocosmospora solani*, *P. citrinum* and *P. brevicompactum*. In general, there was a higher occurrence of *Penicillium* spp., *Fusarium* spp. and *Trichoderma* spp., and the predominant species were *F. fabacearum* and *P. citrinum*. The mycobiota of Brazilian cassava proved to be extremely diverse, and the occurrence of several species in cassava tubers and/or products are reported herein for the first time. Potentially toxigenic species were found in cassava tubers, cassava products and soil, showing how important it is to constantly monitor these substrates.

1. Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the Euphorbiaceae family. It is a perennial shrub capable of adapting to a range of edaphoclimatic conditions and is cultivated in tropical and subtropical regions, especially in developing countries, where it presents itself as one of the best sources of low-cost carbohydrates (El-Sharkawy, 2004; Li et al., 2017). Brazil is the biggest cassava producer in the western hemisphere and ranks fourth in the world. Nigeria is the leading producer (FAOSTAT – Food and Agriculture Organization of the United Nations, 2022), where it is grown by small farmers for their own consumption, and any surplus is marketed as a cash crop (FAO – Food and Agriculture Organization of the United Nations, 2000).

After the cassava harvest, the tubers should be stored for no >2–3 days (Kouakou et al., 2016) to avoid rapid deterioration, and for this reason, they are sold for fresh consumption or processed to add commercial value.

Moist starch extracted from cassava tuber is fermented naturally. This is a traditional technology widely used in Latin America to produce cassava starch, sour starch and tapioca flour. Sour starch processing results in higher acidity. Initially, the tuber is cleaned, peeled and grated, regardless of the end product. The resulting dough is washed in very fine mesh sieves and pressed until the water is transparent, indicating that all the starch has been removed. The dough is used to produce cassava flour and the filtrate to obtain starch. It is then sieved and oven-dried under constant stirring, and then roasted to produce its characteristic flavor (Ono and Taniwaki, 2021).

The cassava starch and sour starch are obtained by placing the filtrate in decantation tanks. For cassava starch, this stage lasts 18 to 24 h, allowing the starch to settle at the bottom of the tank. The residue is

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https://doi.org/10.1016/j.ijfoodmicro.2022.109909
Received 5 April 2022; Received in revised form 18 August 2022; Accepted 26 August 2022
Available online 2 September 2022

then removed and sun-dried for about 8 h until the moisture content drops to 13–14 %. Cassava starch is the most refined product extracted from cassava tubers and is widely used in the food, pharmaceutical, and plastics industries. Tapioca flour is produced by re-hydrating cassava starch (Ono and Taniwaki, 2021).

To produce sour starch the decanting stage is longer. The starch remains in the fermentation tanks for 15 to 40 days. Bacteria and yeasts transform part of the starch into lactic acid, which increases the acidity of the end product. Finally, the residue is removed from the tanks and dried in the sun (Ono and Taniwaki, 2021).

Filamentous fungi are a major concern in cassava cropping. Root rot is one of the main diseases that affect cassava in the field. It impairs yield, inflicting significant economic losses (Gomes and Leal, 2003).

Root rot has been attributed to several fungal genera and the main causal agent is still to be identified, although there is a consensus that filamentous fungi are responsible. Another concern regarding filamentous fungi is the occurrence of toxigenic species and/or mycotoxins; potentially toxigenic species such as *Aspergillus flavus* have been found in cassava tubers, while aflatoxins have been reported in cassava products (Adjovi et al., 2014; Aghimien and Ikenebomeh, 2017; Kaaya and Eboku, 2010; Manjula et al., 2009).

Cassava crop contamination by filamentous fungi can occur in the field and during post-harvest. In the field, factors inherent in cassava cropping, such as hot and humid climatic conditions, high carbohydrate content and contact with the soil (the source of numerous fungal groups), are factors that contribute to contamination (Adjovi et al., 2015). During post-harvest, the processing technology leaves much to be desired, since it is implemented mostly by small industrial cooperatives that often employ semi-artisanal production models, resulting in conditions favorable to fungal proliferation and the production of mycotoxins, especially during starch fermentation (Bankole and Adebanjo, 2003; Dósea et al., 2010).

Studies investigating cassava mycobiota are scarce in the literature. Since Brazil is one of the leading cassava producers and exporters, studies are crucial for understanding and taking preventive and corrective measures along the production chain. Therefore, the aim of this study was to investigate the mycobiota in cassava and derived products in Brazil, focusing mainly on potentially toxigenic groups.

2. Materials and methods

2.1. Samples

A total of 79 samples were analyzed: cassava tubers ($n = 27$), tapioca flour ($n = 9$), cassava flour ($n = 23$), cassava starch ($n = 11$) and sour starch ($n = 9$). These samples were collected from small farms and factories in Assis, Cândido Mota, São Pedro do Turvo, Campos Novos Paulista, Ribeirão do Sul, Palmital and in Campinas city markets, all located in São Paulo state (Brazil). In addition, we also evaluated 22 samples of cassava cropping soil collected in the aforementioned towns.

The amount of cassava tubers collected for each sample varied concerning the number of roots present in each plant and the size of the tubers found. The tubers were pulled out of the ground with the aid of a shovel. Each tuber from the same tree was collected and considered one sample. For the soil samples, after digging about 10 cm deep, approximately 0.5 kg were collected. Each sample of cassava tubers and soil was enclosed in plastic bags, separately, and all samples were kept refrigerated until analyzed.

2.2. Determination of water activity

Approximately, 5 g of each sample was used; enough to cover the capsule. The water activity (a_w) of all samples was determined in triplicate using an Aqualab Series 3TE water activity meter (Decagon, Pullman, WA, USA) at 25 °C ± 1.

2.3. Count and fungal isolation

Cassava tuber samples were directly plated according to Pitt and Hocking (2009). Tubers were peeled and cut into small pieces. The surfaces were disinfected in a 0.4 % sodium hypochlorite solution for 1 min, then 30 pieces were plated on Dichloran Rose Bengal Chloramphenicol agar (DRBC) (10 pieces/plate; three plates 90 × 15 mm). After 5–7 days of incubation at 25 °C, fungal occurrence was determined based on morphological characteristics, and the percentage of infected pieces was counted according to Pitt and Hocking (2009).

Cassava starch, sour starch, cassava flour, tapioca flour and soil samples were analyzed by serial dilution plating. A 25 g subsample from each sample was diluted in 225 mL of peptone water (0.1 % peptone). An aliquot of 0.1 mL was spread-plated onto Dichloran (18 %) Glycerol Agar (DG18) (90 × 15 mm plates) and incubated at 25 °C for 5–7 days. The number of colony forming units (CFU) was then counted and fungal incidence determined based on morphological characteristics (Pitt and Hocking, 2009).

2.4. Morphological analysis

The fungi were evaluated according to their microscopic and macroscopic characteristics. All fungal isolates ($n = 493$) were purified and inoculated at three equidistant points onto Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA) and Yeast Extract Sucrose Agar (YESA) at 25 °C and left for 5 days.

The fungal groups were characterized morphologically, according to taxonomic keys by Samson and Pitt (2000), Klich (2002) and Pitt and Hocking (2009), supplemented with other sources when necessary. Morphological identification was restricted to the level of genus/fungal group (morphogroups) and was combined with molecular analysis.

2.5. Molecular analysis

Based on the morphological analysis, 60 isolates were selected for molecular identification at the species level, the choice of these representatives was based on the morphogroups found in the previous step.

To extract genomic DNA, the purified strains were grown in a liquid medium of yeast sucrose (YES) at 25 °C for 3 days until a mycelial film was formed, and then macerated using liquid nitrogen. This material was used to obtain genomic DNA (PureLink Plant kit, Invitrogen, USA) according to the manufacturer's protocol. The DNA was quantified by spectrophotometry (NanoDrop®, Thermo Scientific).

For molecular identification, different loci were amplified according to the fungal group. For isolates identified morphologically as *Fusarium* spp., the translation elongation factor (*TEF-1α*) was used, based on the primers described in O'donnel et al. (1998). For fungi identified morphologically as *Penicillium* spp., the beta-tubulin locus (*BenA*) was used, based on the primers described in Glass and Donaldson (1995). For fungi identified morphologically as *Trichoderma* spp., the RNA polymerase II second largest subunit locus (*RPB2*) was used, based on the primers described in Houbraken et al. (2012a), and for all other fungal groups, the universal fungi barcode, ITS region (*rDNA*), was used, based on the primers described in White et al. (1990). Amplification conditions were as described in Silva et al. (2020) and Watanabe et al. (2011).

PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific, UK) according to the manufacturer's protocol. Amplicons were sequenced in both directions (forward and reverse) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in a SeqStudio Genetic Analyzer (Applied Biosystems, USA).

The sequences obtained were compared by local alignment using the BLAST tool (Altschul et al., 1990) against the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and MYCOBANK MLST databases (https://www.mycobank.org/page/Pairwise_alignment). In addition, phylogenetic trees were inferred based on Maximum Likelihood (ML). The best nucleotide substitution model was selected for each alignment based on

Table 1
Frequency of occurrence (FO), water activity (a_w) and average of fungal infection in cassava tubers.

	Cassava tubers (n = 27 samples)		
Mean a_w = 0.99			
Fungal groups	FO (%)	Average of infection (%)	Range (%)
Absidia spp.	14.8	1.1	0–16.7
Aspergillus spp.	22.2	2.8	0–33.3
Chaetomium spp.	14.8	2.2	0–33.3
Macrophomina spp.	7.4	0.3	0–3.3
Dematiaceous fungi	25.9	1.6	0–10
Filamentous yeast	3.7	1.6	0–43.3
Fusarium spp.	55.6	7.0	0–36.7
Geotrichum spp.	29.6	7.3	0–63.3
Mucor spp.	22.2	1.1	0–10
Nigrospora spp.	7.4	0.6	0–13.3
Paecilomyces spp.	3.7	0.2	0–3.3
Penicillium spp.	48.2	6.2	0–96.7
Pseudohielavia spp.	15.5	1.7	0–23.3
Rhizopus spp.	3.7	0.5	0–13.3
Sordaria spp.	3.7	0.1	0–3.3
Syncephalastrum spp.	3.7	0.4	0–10
Trichoderma spp.	51.9	13.6	0–96.7
Other not identified fungi	3.7	0.1	0–3.3

FO = Frequency of occurrence (number of samples that contained a fungal group/total samples analyzed). Average of infection = Sum of infection values by genus or fungal group in the samples/total samples analyzed.

Akaike Information Criteria (AIC) (Burnham and Andersonl, 1992) (previously calculated in MEGA7 and jModelTest2). Bootstrap analysis was run on 1000 replicates to estimate the level of support for the ML trees, which were constructed in MEGA 7 (Kumar et al., 2016).

3. Results and discussion

3.1. Cassava tubers

All samples of cassava tubers showed fungal infection (27/27). The mean of total infection was 86.79 %, with minimum and maximum infection of 50 % and 100 % respectively. A total of 287 fungi were isolated, from 18 different genera/groups, as shown in Table 1. The predominant genera were *Fusarium* spp. (55.56 %), *Trichoderma* spp. (51.82 %) and *Penicillium* spp. (48.15 %). Other frequent groups were *Geotrichum* spp. (29.63 %), dematiaceous fungi (25.93 %), *Mucor* spp. (22.22 %) and *Aspergillus* spp. (22.22 %) (Table 1).

According to Gomes and Leal (2003), root rot is a disease that affects and severely impairs cassava yield, causing an average drop of 30 %. *Fusarium* spp. has been reported as the main group responsible for this disease (Bandyopadhyay et al., 2006). However, other species such as *Macrophomina phaseolina, Phytophthora nicotianae, Phytophthora drechsleri, Pythium scleroteichum, Neoscytalidium hyalinum* and *Botryodiplodia theobromae* have also been associated with this disease (Bandyopadhyay et al., 2006; Boas et al., 2017; Zinsou et al., 2017).

In a pioneering study, Noon and Booth (1977) investigated the mycobiota of cassava tubers in Colombia. The main genera reported were *Aspergillus, Botryodiplodia, Fusarium, Mucor, Penicillium, Rhizopus* and *Trichoderma*. In Africa, Ngobisa et al. (2015) carried out similar research and reported *Colletotrichum* sp., *Fusarium* sp., *Pestalotia* sp., *Geotrichum* sp., *Sphaerostilberepens, Trichoderma viride* and *B. theobromae*. In Brazil, Boas et al. (2017) investigated samples of cassava tubers with symptoms of root rot and found several fungal complexes (*Fusarium, Lasiodiplodia, Phytophthora, Corallomycetella, Neoscytalidium* and *Diaporthe/Phomopsis*). The literature and our findings show that there is considerable biodiversity in the cassava crop, highlighting the genera (*Fusarium, Penicillium, Mucor, Aspergillus, Trichoderma, Botryodiplodia* and *Phytophthora*).

Using molecular analysis, 19 different species were identified in cassava tubers. Among the *Fusarium* spp. isolated from tubers, the main species found was *Fusarium fabacearum*. In addition, *Fusarium mundagurra* and *Fusarium foetens* were found (Fig. 1).

Fusarium fabacearum and *F. foetens* are classified in the *Fusarim oxysporum* Species Complex. *F. fabacearum* was described in 2019 (Lombard et al., 2019), originally isolated from soybeans and later found in corn, in the rhizosphere of *Pogonarthria squarrosa*, and as a pathogen of *Syzygium malaccensis* (an Indian fruit) in Brazil (Farias et al., 2021; Gryzenhout et al., 2020).

Fusarium foetens has been frequently reported in plants of the Begoniaceae family (Saurat et al., 2013; Schroers et al., 2004; Tian et al., 2010). In 2019, it was first reported as a potentially toxigenic species, producing beauvericin and fusaric acid (González-Jartín et al., 2019).

Fusarium mundagurra is a species belonging to the *Fusarium fujikuroi* Species Complex, a group that includes several mycotoxin-producing species. *F. mundagurra*, was described by Laurence et al. (2016) in soil samples in Australia, and has recently been reported as a producer of fumonisins (Wigmann et al., 2020).

It is worth noting that the *Neocosmospora* genus (formerly *Fusarium Solani* Species Complex) was recently split from *Fusarium* (Crous et al., 2021). In our study, *Neocosmospora solani* (*syn. Neocosmospora rubicola)* was identified as a frequent in cassava tubers. We also identified *Neocosmospora oblonga,* although at lower frequency (Fig. 1). *Neocosmospora* spp. are commonly found in soil, plant debris, living plants, air and water (Sandoval-Denis et al., 2019). *N. solani* has been associated with plant roots as an endophytic and phytopathogenic fungus (Kim et al., 2017; Zheng et al., 2018).

Among the *Trichoderma* spp., the most frequent species found in tubers were *Trichoderma afroharzianum* and *Trichoderma peberdyi*. Other species identified were *Trichoderma koningiopsis, Trichoderma arenarium*, *Trichoderma pseudoasperelloides* and *Trichoderma azevedoi* (Fig. 2).

Trichoderma peberdyi and *T. azevedoi* are species recently described in soil samples from garlic and onion crops in Brazil (Inglis et al., 2020). We found both species in cassava tubers, which suggests that they frequently occur in Brazilian territory. Inglis et al. (2020) also reported the occurrence of *T. afroharzianum,* this species has been identified as the cause of ear rot in maize, in Europe (Pfordt et al., 2020).

Trichoderma arenarium is a halotolerant species isolated from saline soils in China. It has shown potential as a biocontrol agent against phytopathogenic species and as a plant growth-promoting agent (Ding et al., 2020). *T. koningiopsis* has also been investigated as a biocontrol agent against *F. oxysporum* in *Pinus massoniana* (Yu and Luo, 2020), and has been reported as a frequent cosmopolitan species in several tropical plants (Samuels et al., 2006). *T. pseudoasperelloides* was recently described by Zheng et al. (2021), which originally isolated it as endophyte from *Myriophyllum spicatum* leaves, moreover, it was also found in the rhizosphere of tobacco plants.

Among the isolates of *Penicillium* spp. found in tubers, *Penicillium citrinum* was the prevalent species. *Penicillium javanicum* and *Penicillium brevicompactum* were also found (Fig. 3). *P. citrinum* is widely distributed throughout the world and has been isolated from cereals, flours, soil, indoor environments, cocoa, rice, nuts, and pepper/spices, the roots of *Ixeris repens*, and as an endophyte in coffee trees (Houbraken et al., 2010a; Pitt and Hocking, 2009). *P. citrinum* is also a potential producer of citrinin, a nephrotoxic mycotoxin.

Other fungal species found include *Macrophomina pseudophaseolina, Mucor lusitanicus, Neurospora* sp., *Nigrospora* sp. and *Pseudothielavia terricola* (Supplementary Fig. S1).

Many species found herein are reported for the first time in cassava tubers, and several of these species have been described only recently. This fact shows how little in-depth research has been conducted on cassava mycobiota. The species reported herein are closely associated with the soil and/or rhizosphere, and their occurrence in cassava tubers is easily understood.

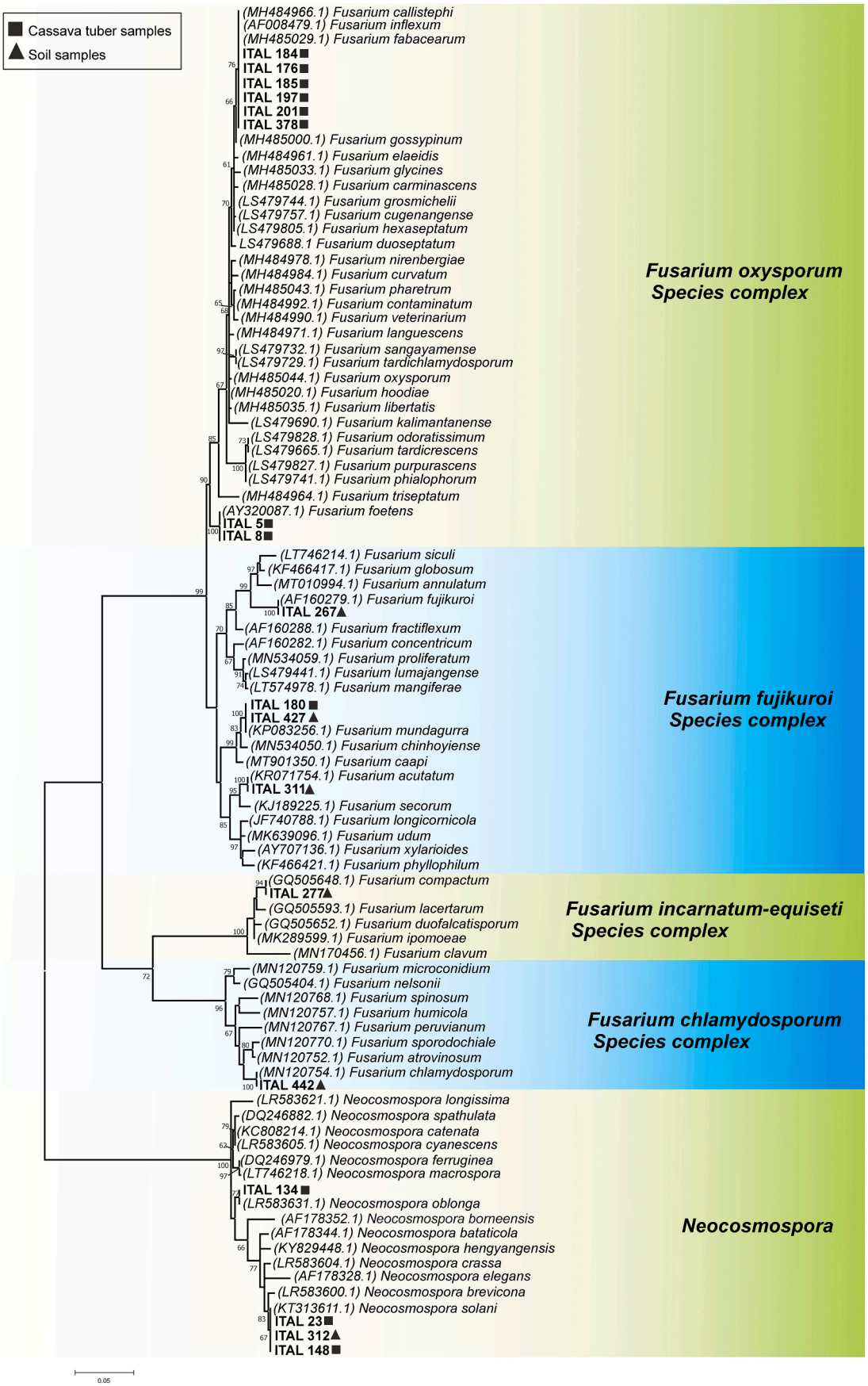

Fig. 1. Maximum likelihood tree (GTR + I + G) based on *TEF-1a* sequences showing the relationships between *Fusarium* spp. and isolates from cassava tubers and soil samples. Bootstrap values (BS) higher than 60 % are shown. Isolates from this study are in bold.

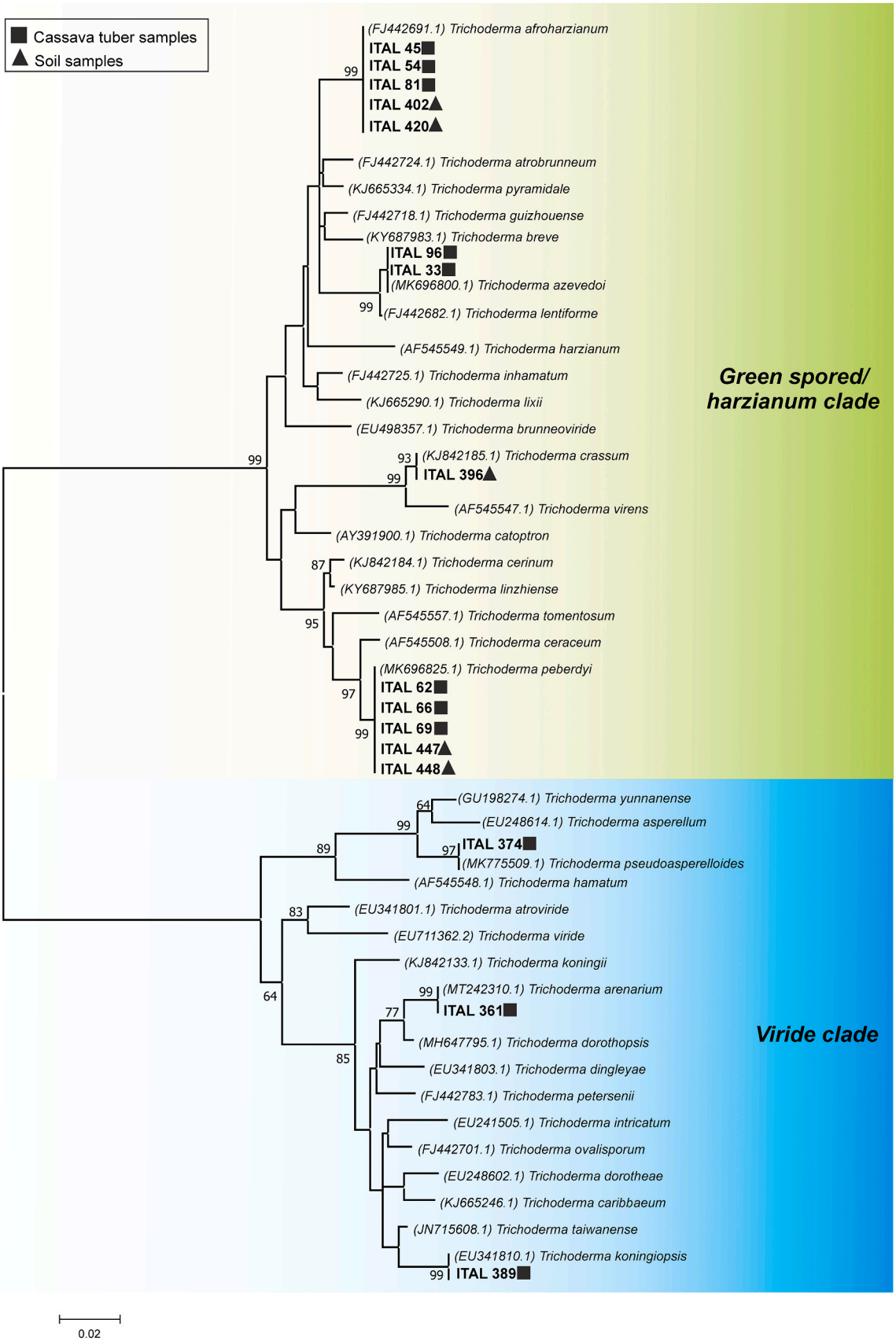

Fig. 2. Maximum likelihood tree (T93 + G) based on *RPB2* sequences showing the relationships between *Trichoderma* spp. and isolates from cassava tubers and soil samples. Bootstrap values (BS) higher than 60 % are shown. Isolates from this study are in bold.

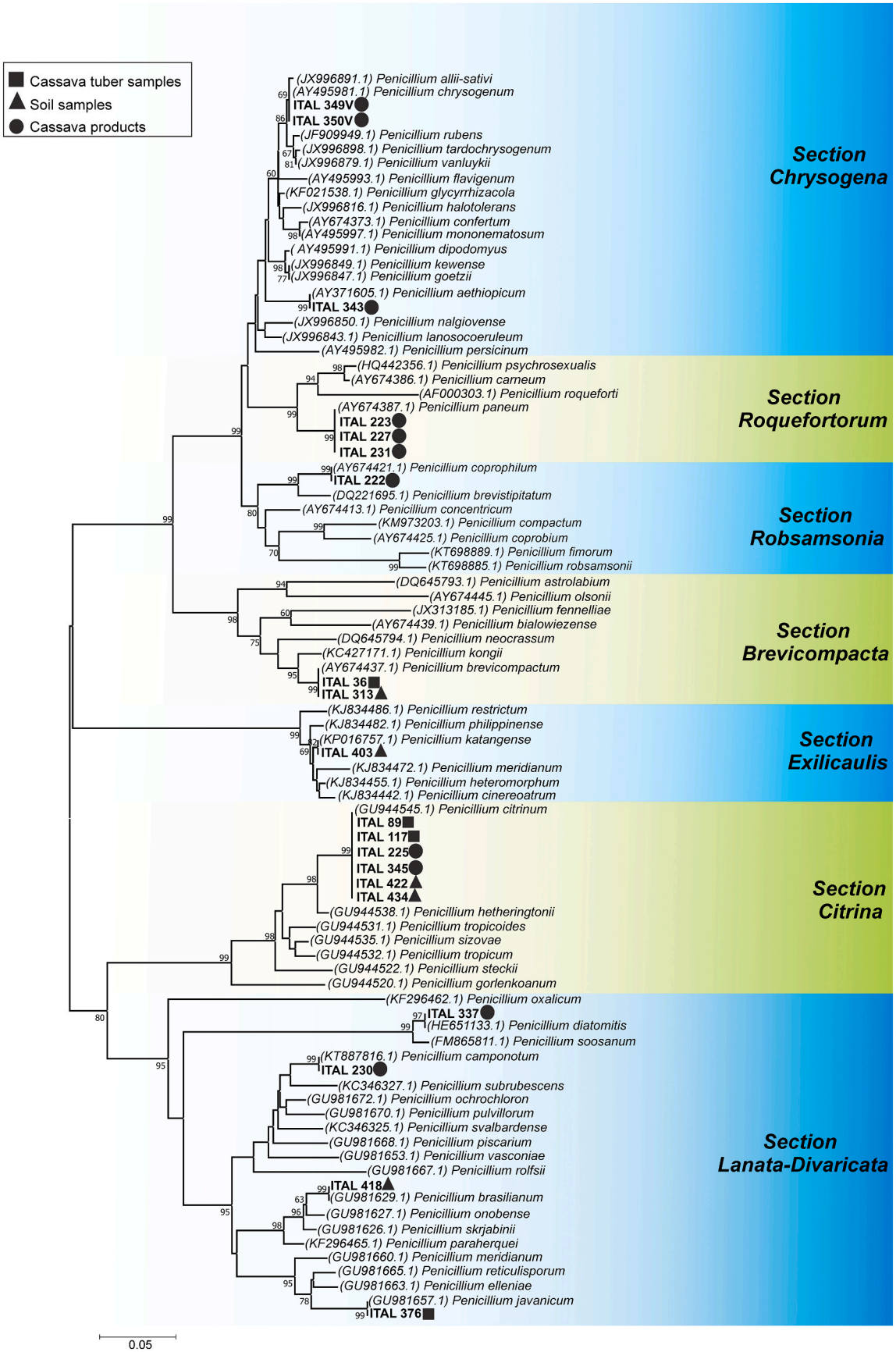

Fig. 3. Maximum likelihood tree (GTR + G) based on *BenA* sequences showing the relationships between *Penicillium* spp. and isolates from cassava tubers and soil samples. Bootstrap values (BS) higher than 60 % are shown. Isolates from this study are in bold.

Table 2
Frequency of occurrence (FO), water activity (a_w) and count for genera or fungal groups found in cassava products.

Sample (number of samples)	Cassava flour (n = 23)			Cassava starch (n = 11)			Sour starch (n = 9)			Tapioca flour (n = 9)		
Mean a_w	0.39			0.46			0.51			0.99		
Fungal groups	FO (%)	Average count (CFU/g)	Range (CFU/g)	FO (%)	Average count (CFU/g)	Range (CFU/g)	FO (%)	Average count (CFU/g)	Range (CFU/g)	FO (%)	Average count (CFU/g)	Range (CFU/g)
Aspergillus spp.	4.4	4.4	0–1.0 × 10^2	18.2	5.5 × 10	0–4.0 × 10^2	0	0	0	0	0	0
Paecilomyces spp.	4.4	4.4	0–1.0 × 10^2	36.4	2.3 × 10^2	0–8.0 × 10^2	22.2	2.2 × 10	0–1.0 × 10^2	22.2	4.6 × 10^3	0–4.1 × 10^4
Cladosporium spp.	0	0	0	9.1	9.1	0–1.0 × 10^2	0	0	0	0	0	0
Dematiaceous fungi	0	0	0	9.1	9.1	0–1.0 × 10^2	0	0	0	0	0	0
Penicillium spp.	8.7	1.3 × 10	0–2.0 × 10^2	36.4	2.3 × 10^2	0–1.3 × 10^3	0	0	0	0	0	0
Rhizopus spp.	0	0	0	9.1	9.1	0–1.0 × 10^2	0	0	0	0	0	0

FO = Frequency of occurrence (number of samples that contained a fungal species/total samples analyzed). CFU/g = Colony Forming Units per gram. Average count = Sum of CFUs counted for the genera or fungal group in the samples/total samples analyzed.

3.2. Cassava products

The cassava products (tapioca flour, cassava flour, cassava starch and sour starch) harbored mainly xerophilic fungi (capable of growing in water activity below 0.85), such as the *Aspergillus, Penicillium* and *Paecilomyces* genera (Pitt and Hocking, 2009) (Table 2). All products exhibited low a_w, with the exception of tapioca flour (a_w 0.99). However, note that tapioca flour processing includes a drying stage that significantly reduces a_w, and it is then rehydrated.

The average fungal count for cassava flour samples was 2.6 × 10 CFU/g. Only 17.4 % (4/23) of the samples were contaminated by fungi. The fungal groups found were *Aspergillus, Paecilomyces* and *Penicillium* (Table 2).

The average fungal count for cassava starch samples was 7.2 × 10^2 CFU/g. Some 54.5 % (6/11) of the samples were contaminated by fungi. The most frequent fungal groups were *Paecilomyces* and *Penicillium* (Table 2).

In sour starch samples, the average fungal count was 3.3 × 10 CFU/g and only 33.3 % (3/9) of the samples were contaminated by fungi. In contrast, some 54.5 % (6/11) of tapioca flour samples were contaminated by fungi and the average fungal count was 4.6 × 10^3 CFU/g. Both sample types were found to contain only *Paecilomyces* spp. (Table 2).

Nine different species in cassava products were identified by molecular analysis. For *Penicillium* spp., the main species found were *Penicillium paneum*, *Penicillium chrysogenum* and *P. citrinum*. Other species were also found, including *Penicillium camponotum* and *Penicillium diatomitis* belonging to section *Lanata-Divaricata; Penicillium aethiopicum* and *Penicillium coprophilum*, belonging to section *Chrysogena* and section *Robsamsonia,* respectively (Fig. 3).

Penicillium paneum belongs to the section *Roquefortorum* and has been frequently reported in animal silage (corn and grass), baked goods, baking yeast, chocolate sauce, stone chambers and cocoa (An et al., 2009; Boysen et al., 1996; Boysen et al., 2000; Moulia et al., 2014; O'brien et al., 2006; O'brien et al., 2008). This species is potentially toxigenic and can produce patulin and roquefortine C (Boysen et al., 1996; Sumarah et al., 2015).

Penicillium chrysogenum is a widely distributed species, colonizing several foods and indoor environments. It can produce secalonic acid D and F and roquefortine C and D (Houbraken et al., 2012b), and was reported in cassava tubers and cassava flour collected in Burundi (East Africa) by Muninbazi and Bullerman (1996). *P. chrysogenum* is a known producer of penicillin (Laich et al., 2002).

Two species of *Paecilomyces* spp. were identified (Supplementary Fig. S1): *Paecilomyces saturatus* (syn. *Paecilomyces dactylethromorphus)* and *Paecilomyces formosus*, both belonging to *Paecilomyces variotii* sensu *lato,* which currently comprises five species, namely *Byssochlamys spectabilis* (the sexual status of *P. variotii*), *Paecilomyces brunneolus, Paecilomyces divaricatus, P. formosus* and *P. saturatus* (Houbraken et al., 2010b; Samson et al., 2009).

Paecilomyces (= *Byssochlamys*) species produce ascospores that are heat resistant and can survive lengthy periods of exposure to temperatures above 85 °C. Recent studies have found that the spores of the *Paecilomyces* genus are among the most heat resistant (Van den Brulee et al., 2020). Furthermore, species in this group can also grow at very low levels of oxygen tension and can form pectinolytic enzymes. These three physiological characteristics combined suggest that *Paecilomyces* species can cause severe spoilage in pasteurized and canned fruits. *P. saturatus* can produce patulin and brefeldin A and has been reported in a wide variety of substrates, such as acetic acid, leather, medicines, watercress seeds and clinical sources. *P. formosus* has been isolated in soil, wood, sponge, humans and the environment (Houbraken et al., 2010b; Samson et al., 2009).

Gnonlonfin et al. (2008) investigated cassava chip samples in Benin and reported the occurrence of *A. flavus, P. chrysogenum, Fusarium verticillioides, Phoma sorghina, Nigrospora oryzae, Mucor piriformis* and *Rizhopus oryzae*. In Uganda, Kaaya and Eboku (2010) found higher incidences of the genera *Mucor* and *Rhizopus* during their investigation of cassava chip samples, and also reported the occurrence of *Aspergillus, Penicillium* and *Fusarium*. Similar findings were reported by Jimoh and Kolapo (2008) in Nigeria, who detected *A. flavus, Aspergilus niger, F. oxysporum* and most frequently *Rhizopus nigricans*.

Wareing et al. (2001) analyzed 101 samples of dried cassava (called "kokonte" in Ghana), and reported the occurrence of *Aspergillus, Alternaria, Colletotrichum, Drechslera, Fusarium, Monilia, N. oryzae, P. sorghina, Geotrichum, Aureobasidium, Mucor, Rhizopus, Penicillium, P. variotii* and *Wallemia sebi*.

According to the literature, the most frequent genera are *Mucor, Rhizopus, Aspergillus, Penicillium* and *Fusarium*, and there seems to be great diversity. In general, for the samples of cassava products analyzed herein, the most frequent genera were *Penicillium* and *Paecilomyces*. In contrast to the aforementioned studies mentioned above, we did not find *Mucor* and *Fusarium*. However, note that the product analyzed in most of these studies was cassava chips, while the products analyzed in our study are cassava flour, cassava starch, sour starch and tapioca flour, which tend to have distinct physicochemical characteristics and, therefore, the difference in the mycobiota is only to be expected.

Table 3
Frequency of occurrence (FO), water activity (a_w) and counts of genera or fungal groups found in soils cropped with cassava tubers.

	Soil (n = 22 samples)		
Mean a_w = 0.98			
Fungal groups	FO (%)	Average count (CFU/g)	Range (CFU/g)
Aspergillus spp.	40.9	2.0×10^3	$<100–1.6 \times 10^4$
Cladosporium spp.	81.8	5.2×10^3	$<100–2.2 \times 10^4$
Dematiaceous fungi	9.1	9.1×10	$<100–1.0 \times 10^3$
Fusarium spp.	54.6	1.4×10^3	$<100–1.0 \times 10^4$
Paecilomyces spp.	9.1	9.1	$<100–1.0 \times 10^2$
Penicillium spp.	86.4	2.0×10^4	$<100–1.1 \times 10^5$
Pseudothielavia spp.	4.6	4.6×10	$<100–1.0 \times 10^3$
Syncephalastrum spp.	4.6	4.6×10	$<100–1.0 \times 10^3$
Talaromyces spp.	4.6	4.6×10	$<100–1.0 \times 10^3$
Trichoderma spp.	36.4	2.6×10^3	$<100–2.2 \times 10^4$
Other not identified fungi	4.6	4.6	$<100–1.0 \times 10^3$

FO = Frequency of occurrence (number of samples that contained a fungal species/total samples analyzed). CFU/g = Colony Forming Units per gram. Average count = Sum of colony count values for the genera or fungal groups in the samples/total samples analyzed.

3.3. Soil samples

All 22 soil samples exhibited fungal contamination. The average fungal count was 4.1×10^4 CFU/g, ranging between 1.8×10^4 and 1.2×10^5 CFU/g. As can be seen in Table 3, the most frequent genera in the soil samples under cassava were *Penicillium, Cladosporium* and *Fusarium*. Fungi groups found at lower frequency were *Aspergillus* spp., *Paecilomyces* spp., *Syncephalastrum* spp., *Pseudothielavia* spp., *Trichoderma* spp., *Talaromyces* spp. and ascomycetes and dematiaceous fungi.

The *Penicillium* genus was present in over 86 % (19/22) of samples and was the most frequent group found in soil samples (Table 3). Four species of *Penicillium* spp. were identified: *P. brevicompactum, P. citrinum, Penicillium brasilianum,* and *Penicillium katangense* (Fig. 3).

Penicillium brevicompactum has been reported in soil samples (Marchisio et al., 1991), and interestingly in samples from the Antarctic biome (Godinho et al., 2015; Molina-Montenegro et al., 2016; Oses-Pedraza et al., 2020). This species, often found in fruits as a weak pathogen, has been reported to cause spoilage in grapes, mushrooms, apples, potatoes and cassava (Pitt and Hocking, 1997). *P. brasilianum* has been reported mainly in soil (Abbas et al., 2009; Cho et al., 2005; Fujita and Hayashi, 2004; Schurmann et al., 2010). It can produce mycotoxins such as verruculogen and penicilic acid, and has been widely investigated for the production of bioactives of interest (Bazioli et al., 2017).

The following species of *Fusarium* spp. were identified: *Fusarium chlamydosporum*, *Fusarium acutatum*, *Fusarium compactum, F. fujikuroi* and *F. mundagurra*. *N. solani* (formerly FSSC) was also found (Fig. 1). *F. chlamydosporum* is a potential producer of enniantis, moniliformin and chlamydosporol (Munkvold et al., 2021) and is associated with several plants as an endophyte or phytopathogen. It has been frequently reported in soil and the rhizosphere (Hami et al., 2021; Lazreg et al., 2013; Minati and Mohammed-Ameen, 2019; Siddiquee et al., 2010).

Fusarium fujikuroi is one of the most frequent *Fusarium* spp. species found in cereals, especially rice, (Choi et al., 2018; Naeem et al., 2019; Qiu et al., 2020; Yilmaz et al., 2021). It is associated with contamination by various mycotoxins such as beauvericin, fumonisins, fusaric acid, fusarins and moniliformin (Munkvold et al., 2021). In addition to *F. fujikuroi*, two other species of the *F. fujikuroi* Species Complex were also found: *F. mundagurra* (also found in the tuber samples) and *F. acutatum*, a species capable of producing beauvericin, enniantis, and moniliformin (Munkvold, 2017).

Fusarium compactum is capable of producing enniantis and trichothecenes (Munkvold, 2017), and has been reported in cereals and soil. It has also been found and as a pathogen in banana (Besharati et al., 2017; Frisullo et al., 1994; Manshor et al., 2012; Onyike and Nelson, 1992).

The *Cladosporium* genus, the second most frequently found herein, was detected in 81.8 % (18/22) of samples. This high frequency of occurrence in soil samples corroborates the findings of Bensch et al. (2012), who reported that this group is commonly found in air and soil samples. A single species, *Cladosporium anthropophilum*, was identified in this genus (Supplementary Fig. S1). It is a saprobic fungus described by Sandoval-Denis et al. (2016), and is frequently found in indoor air, food, plants and clinical sources, and can be pathogenic to humans (Bensch et al., 2018). This species was recently found in decayed leaves in Brazil (Freitas et al., 2021).

Trichoderma spp. were also present in the soil samples (Table 3); the species found were *T. afroharzianum, T. peberdyi*, also present in cassava tubers. Moreover, we also identified *Trichoderma crassum* (Fig. 2).

Other species identified in soil samples were *Aspergillus brasiliensis* and *Aspergillus wentii* (Supplementary Fig. S1).

Sule and Oyeyiola (2012) studied the mycobiota of cassava soils in Nigeria and reported the presence of *Aspergillus, Brettanomyces, Botrytis, Byssochamys, Doratomyces, Geotrichum, Gliocladium, Moniliella, Mucor, Monascus, Neurospora, Oidiodendronia, Penicillium, Piricillium, Penicillium Rhodotorula, Rhizopus, Saccharomyces, Cladosporium, Humicola, Trichoderma, Ustilago, Acremonium* and *Trichophyton.*

Soil can serve as a natural source of infections for fungal species (Perrone et al., 2007; Winter and Pereg, 2019); in our study, some species, such as: *T. afroharzianum*, *T. peberdy*, *N. solani*, *F. mundagurra* and *P. brevicompactum*, were present in the soil and also in the cassava tubers. As already mentioned, many of these species are commonly found in soils and tubers (Inglis et al., 2020; Kim et al., 2017; Laurence et al., 2016; Marchisio et al., 1991).

Among all the species identified here, *P. citrinum* was the only one that occurred in all the cassava production chain, from the soil to the final product (Fig. 3), which is worrying, since it is a potentially toxigenic species.

4. Conclusions

The mycobiota of cassava tubers, cassava products, and soil is highly diverse. A total of 20 fungal groups were morphologically characterized and 37 different species were molecularly identified, and at least 16 species (*F. fabacearum, F. foetens, F. mundagurra, N. oblonga; T. afroharzianum; T. azevedoi, T. peberdy, T. pseudoasperelloides, T. kongipois, T. arenarium; P. diatomitis, P. camponatum, P. copropylum; Mucor lusitanicus; P. saturatus* and *P. formosus*) were reported for the first time in cassava and/or its products. In general, the most important fungal groups were *Penicillium* spp., *Trichoderma* spp. and *Fusarium* spp.. Nine potentially toxigenic species were found, some of them were present in the soil and cassava samples, a fact that denotes the importance of constant monitoring of the mycobiota in these substrates. For the first time, we reported the occurrence of *P. saturatus* and *F. mundagurra* in cassava and/or its products, species potentially producing patulin and beauvericin, respectively. In addition, *P. citrinum*, a citrinin-producing species, was confirmed as one of the most frequent in cassava. These results encourage further investigations into the occurrence of these mycotoxins, which have been neglected, in the cassava production chain.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2022.109909.

Funding

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process 2018/25552–0; 2013/05414–8; J. J. Silva grant # 2018/25597–3), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process 305649/014–0), Fundação de Desenvolvimento de Pesquisa do Agronegócio (FUNDEPAG), and Coordenadoria de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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