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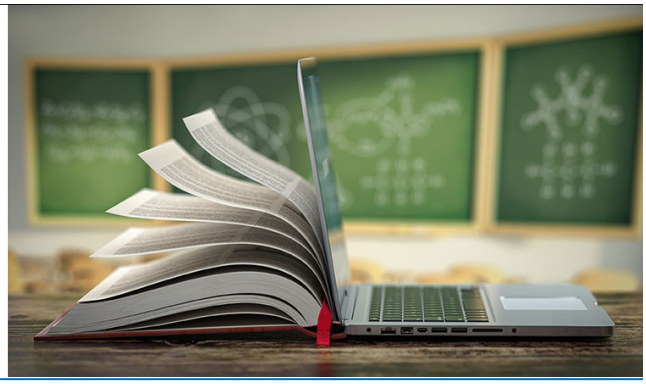

First report on Basidiomycota fungi in sorghum and millet from Southwest Nigeria

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Abstract. Trametes species are mushroom fungi with several biotechnological applications. This includes decolourisation of wastewater from olive mill and elimination of endocrine-disrupting hormones.This study reports the presence of two Trametesspecies, namely Trametespolyzona and Trametesvillosa from the phylumBasidiomycota in sorghum and milletvended inSouthwest Nigeria. These fungal isolates were identified culturally and further identified through phylogenetic characterisation. Trametessp occurred in 10% of sorghum samples and 20% of millet samples. The two species were morphologically similar but distantly related phylogenetically. Most fungal species present in cereal crops belong to the division Ascomycota. However, two Trametesspecies belonging to division Basidiomycota are being reported for the first time in cereal crops.Trametessp can be harnessed for their health benefits such as the treatment of cancer and the reduction of viral activity in humans.

Keywords:Trametes species, Basidiomycota, sorghum, millet, Southwest Nigeria

1. Introduction

Sorghum (*Sorghum bicolor*), is the fourth most cultivated cropafter paddy, wheat, and maize in the world [1].Nigeria,which is ranked third among the world producer of sorghum after

UnitedStates of America and India, produces40% of the total sorghum recorded in Africa [2].Millet (*Pennisetumglaucum*) is the sixth most important grain in the world, produced mainly by China, India, and Nigeria [1, 3]. Fungal contamination occurs in these cereals in the field and also during the process of drying, processing, transporting and storage, which thus enhance the production of mycotoxins under favourable conditions [4]. Fungi which colonise cereals include species of *Fusarium, Aspergillus,* and *Penicillium,* which significantly reduce crop yield, quality and safety owing to their capacity to produce mycotoxins [4].Sorghum and millet are among cereals that get contaminated both on the field and during storage [5, 6]. These cereals are staple foods consumed in Southwest Nigeria in the form of fermented gruels such as 'Ogi', fermented drinks such as 'kunu' and snacks such as 'donwka' and 'kokoro'. Oranusi*et al.* [6] attributed the presence of fungi in cereal grains to the normal flora of the plants as well as contamination of the grains from the field or during storage and display for sales.

2. Sample collection

One hundred samples each of sorghum and millet were collected from the six states in the South-west of Nigeria, namely: Lagos, Ogun, Osun, Ondo, Ekiti and Oyo. After that, twenty composite samples each of sorghum and millet were separately prepared for the isolation of the fungal species.

3. Materials and Methods

3.1 Isolation of Fungi from the cereal grains

One gram of each ground composite grain sample was homogenised in 9 mL of sterile 0.1% peptone water solution, vortexed and serial dilutions to 10^{-10} were carried out.Aliquots of the various dilutions were inoculated onto freshly prepared Potato Dextrose Agar plates and incubated for 5 days at 25°C. The inoculated plates were observed for fungal colonies after 5 days of incubation. Distinct colonies from the culture plates were subcultured onto fresh Czapek Yeast Extract agar plates and incubated at 25°C for 5 days. The identification of pure isolates was carried out based on their macroscopic and microscopic features according to the keys of Pitt and Hockings[7].

3.2 Genomic DNA Extraction

Fresh mycelium from purified culture was collected in sterile 2 mL Eppendorf tubes. Approximately 500 µl of cetyltrimethylammonium bromide (CTAB) (1 M Tris–HCl, pH 8.0), 0.5M EDTA, 5M NaCl, 20 g CTAB) buffer was added to the tubes. Mycelium was then ground against the wall of the tube using sterile pipette tips for genomic DNA (gDNA) isolation using the method described by Aamir *et al.* [8]. A Nanodrop spectrophotometer was used to quantify the DNA of the fungi.

3.3Polymerase Chain Reaction (PCR) Analysis

The isolated DNA was subjected to Polymerase Chain Reaction (Veriti thermal cycler, Applied Biosystems) as described by Aamir *et al.* [8]. Isolates were putatively identified by analysis of sequences of the Internal Transcribed Spacer (ITS) gene. These sequences were amplified using the primers ITS1 F (TCCGTAGGTGAACCTGCGG) and ITS4 R (TCCTCCGCTTATTGATATGC). Amplicons were confirmed with gel electrophoresis using a 1% agarose gel. The PCR products were then purified with gel filtration columns loaded with 6% Sephadex G-50 (50-150 µm bead size) (Sigma).

3.4 Sequencing of PCR Products

The purified PCR products were sequenced using forward and reverse primers with ITS1 and ITS4 primers, respectively, using the ABI Prism™ 3500 x 1 Genetic Analyzer (Applied Biosystems, CA, USA). The ABI Prism™Bigdye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used with protocols supplied by the manufacturer.

3.5 Sequence Data Analysis

Sequence reads generated from ITS1, and ITS4 primers were assembled into consensus sequences using the BioEdit software package. These sequences were then compared to those in the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) nucleotide database using BLAST searches. The putative identity of isolates was based on the highest similarity score to the newly produced sequences.

3.6 Phylogenetic Analyses

The evolutionary relationship of fungi isolated from maize, sorghum and millet were obtained from a Neighbour-Joining analysis as reported in Casa*et al.* [9]. The evolutionary distances were determined by the Maximum Composite Likelihood method, as referenced by Tamura*et al.*[10]. The phylogenetic and molecular evolution analyses were conducted using MEGA X Software referenced by Kumar *et al.*[11].

4. Results and Discussion

The phylogenetic tree for the *Trametes* species which was grouped into six clades revealed that *Trametespolyzona*MKO20685 was distantly related to other species such as *Trametespolyzona* voucher_OH272sp, *Trametespolyzona* strain DMC367, *Trametespolyzona* voucher BKW004 and *Neofomitellapolyzonata* voucher Dai_10980 all in clade 1 as shown in Fig. 2. *Trametesvillosa*MKO20684 was distantly related to *Trametesvillosa* culture collection_BRFMFRA_1375, *Trametesvillosa* strain CBS_334.49, *Trametesvillosa* strain_Sc10 and *Trametesvillosa*isolate_P4. Two different species of *Trametes* were isolated from millet in this study. These are *Trametesvillosa* and *Trametespolyzona* (Plate 1 and 2, respectively).

There is a lack of information on the presence of *Trametespolyzona* and *T. villosa* in cereals. However, these *Trametes* species were isolated in some sorghum and millet samples in this study.*Trametes* species have been reported in forests and dead and decaying hardwood [12]; thus, it may be considered as one of the field fungi in cereals.*Trametespolyzona,* previously named *Coriolopsispolyzona*, was first described as Basionym *Polyporuspolyzonus* [13]. *Trametes* species isolated in the sorghum and millet grains were most likely introduced as spores from the field where the cereals were grown before the grains were bagged and transported for sale in the markets. Cereal grains for sale in the market are usually kept in stores away from moisture until they are sold. However, these grains may also be affected by climatic conditions such as the temperature of the storage environment, which is temperate. Several fungi in grains are pathogenic and are mycotoxin producers.

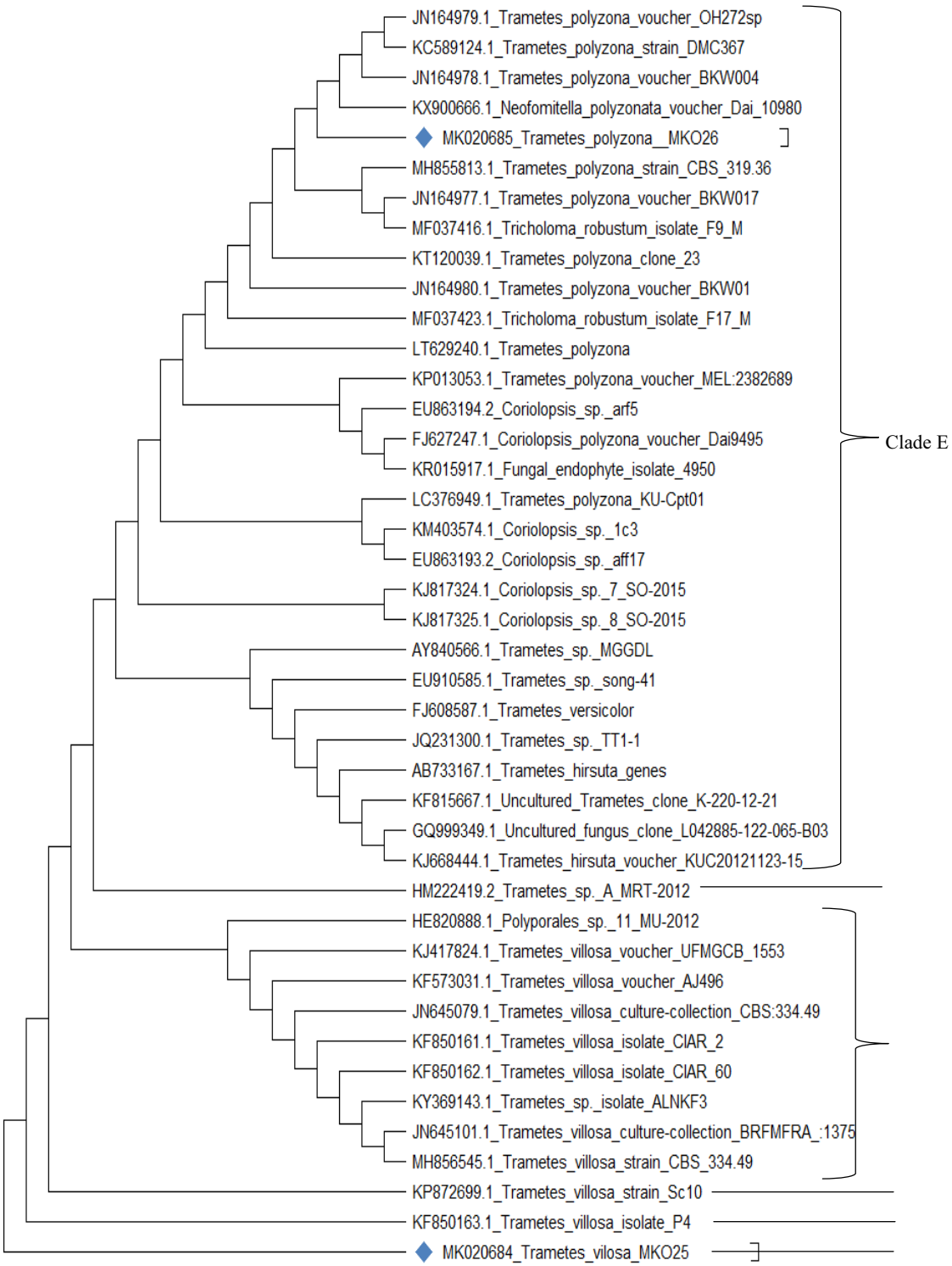

Figure 1: Phylogenetic tree of *Trametes*sp created by the Neighbour-joining Method

Therefore, various measures have been employed to ensure food sustainability such as fungal decontamination methods using *Cymbopogon citratus*in maize [14] and mycotoxin decontamination methods in cereal grains using a combination of Montmorillonite clay and *Cymbopogon citratus*[15, 16]. However, the presence of *Trametes* sp in cereals such as sorghum and millet may not pose a health risk as they have not been reported to produce mycotoxins but rather have potential uses in medicine and the industry.

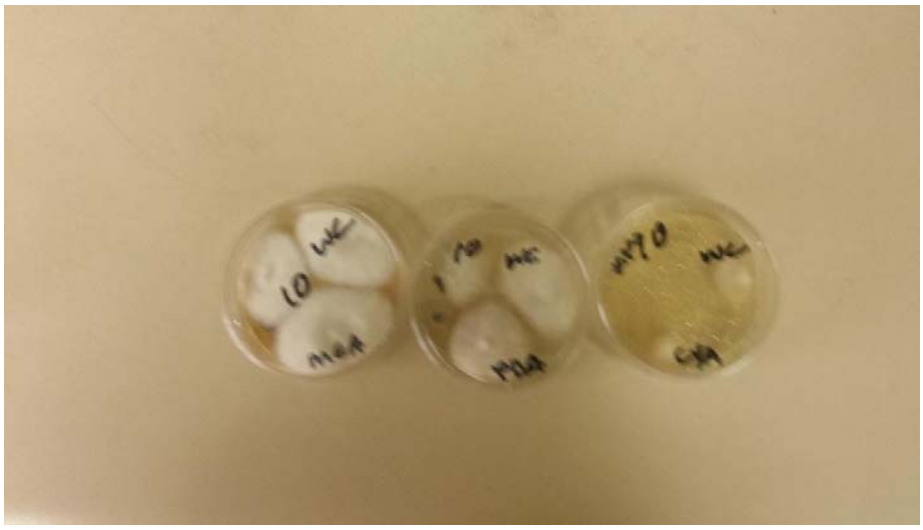

Plate 1: *Trametesvillosa*on Malt Extract Agar, Potato DextroseAgar and CzapekYeast Agar

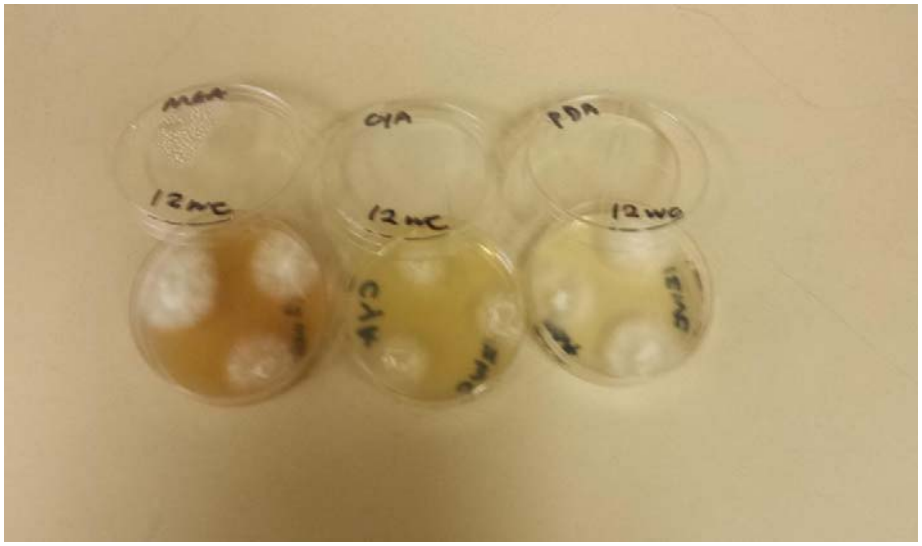

Plate 2: *Trametespolyzona*on Malt Extract Agar, CzapekYeast Agar and Potato Dextrose Agar

5. Conclusion and Recommendation

This study documents for the first time, the occurrence of *Trametes*sp in cereals from Southwest Nigeria. *Trametes* species have biotechnological potentials in bioremediation and medicine. Hence, it can be beneficialinthe economic development of the nation. Cereal grains across other geopolitical zones in Nigeria should be screened for the presence of *Trametes* species.

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