

Molecular identification of fungi associated with fig-mulberry (*Ficus sycomorus* L.) fruits in Nigeria

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Abstract

The fruit of *Ficus sycomorus* L. holds significant economic value in Nigeria. It offers a myriad of medicinal advantages. Despite the plant's importance, it has been noted that the shelf-life of its fruits is compromised by various diseases caused by fungal pathogens. This study was conducted to isolate and identify the prevalent fungal pathogen responsible for the decay of *F. sycomorus* fruits. Diseased fruits were collected from Okochiri in the Okrika Local Government Area of Rivers State and were identified morphologically. The DNA of the most frequently isolated fungi, coded FF2 9-7 and FF2 5-8, were characterized molecularly using Internal Transcribed Spacer (ITS-1 and 2) markers. The morphological analysis revealed that the FF2 9-7 and FF2 5-8 isolates belonged to the *Pestalotiopsis* and *Dipodascus* genera. The molecular weight of the isolate's DNA exceeded 500 base pairs. According to sequence similarity, the DNA sequence of the isolate was found to be 80.83% identical to that of the *Pestalotiopsis mangiferae* strain and 97.06% identical to *Dipodascus* sp. strain F1 E06. Consequently, these findings indicate that *Pestalotiopsis mangiferae* and *Dipodascus* sp. are the fungal pathogens responsible for post-harvest rot in *F. sycomorus* fruits. It is anticipated that these results will contribute to the development of effective disease management strategies to reduce ill effects of fungi invading fig-mulberry fruits while also laying the groundwork for further investigation into the potential mycotoxin effects of consuming infected *F. sycomorus* fruits.

Keywords: Ficus fruits, Fungal pathogens, *Dipodascus* sp, DNA, *Pestalotiopsis mangiferae*.

Introduction

Ficus sycomorus is a kind of fig that has been grown since ancient times. It is also known as the sycamore fig, the fig-mulberry because the leaves look like those of the mulberry (Zohary and Hopf, 2012). They belong to Moraceae, a family that is reputed for its medicinal qualities and composed of roughly 40 genera and over 1,400 species of trees, shrubs, vines and plants, frequently with milky latex fluids that have been cultivated since ancient times (Zerega *et al.*, 2005). *Ficus sycomorus* relies on specific wasps from the Agaonidae family for pollination, and these wasps can only develop within the fig's gall flowers, creating a mutual dependency between the plant and the wasps (Cook and West, 2005).

Ficus sycomorus has large edible fruits of about 2–3 cm in diameter (Afaf *et al.*, 2015), that turn from buff-green to yellow or red when ripe (Singh *et al.*, 2011). They grow in thick clusters on long branchlets or leaf axils, with flowering and fruiting happening throughout the year, especially from July to December. Fruit production happens year-round, starting in April (or later, depending on the variety) and lasting through the rest of the year. *Ficus sycomorus* fruits are low in fat and cholesterol, and packed with vitamins, and rich in dietary fiber (Slavin, 2006) and mineral elements. Fruits are used

in medicine for the treatment of diabetes (Choo *et al.*, 2012; Farsi *et al.*, 2013) and gastrointestinal tract issues like bloating, diarrhea, and dysentery. Several researches have shown that it has significant antiseptic, antibacterial, and antifungal qualities (Sena *et al.*, 2017). According to Sena *et al.* (2017), it is also used to treat respiratory conditions, including coughs, bronchitis, and asthma. Its little laxative effect is quite useful as a part of the Mediterranean diet. According to the report of Shi and Mon (2018), fresh fig mulberry fruits are rich in pectin, a type of soluble fiber that helps to keep lower cholesterol levels in the human body. It contains a lot of antioxidants, a good source of polyphenols and flavonoids (Yan *et al.*, 2014). It has also been traditionally used for its medicinal benefits as a metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy (Slavin, 2006). Doehlemann *et al.* (2017) noted that fungi use various strategies to colonize plants, leading to outcomes that range from beneficial relationships to host death. *Ficus* plant (Fig. 1) is vulnerable to fungal infections that impact fruit output and quality, much like other fruit crops. Identifying fungal organisms associated with the fruits is an important and obligatory step as it helps plant pathologists make informed decisions in disease control and management to prevent yield

losses. This study was carried out to identify the fungal pathogens associated with *Ficus sycomorus* in Nigeria.

Materials and Methods

Origin of plant samples

Diseased fig-mulberry fruits were obtained in July 2024 from Okochiri Community in Okirika Local Government Area in Rivers State, Nigeria.

Isolation of fungi from diseased fruits

Ficus sycomorus fruits were tested for fungal pathogens using the standard blotter method, as outlined by the International Seed Health Testing Association (ISTA, 2016). Equipment preparation involved autoclaving filter paper, distilled water in a conical flask, and foil-wrapped Petri dishes at 121 °C for 15 min. Sterilized 9 cm Whatman filter papers (3 layers) were placed in Petri dishes, moistened with water, and covered. Ten fruit pieces were surface-decontaminated with 70% ethanol for 2 min, washed two times with germ-free distilled water, and plated (10 pieces per dish). Plates were labelled, sealed with masking tape, and incubated at 25 ± 2 °C for 7 days. Identified fungi were sub-cultured on potato dextrose agar (PDA) at room temperature (25 ± 2 °C) in darkness.

Fungal DNA isolation

DNA extraction was done using the PuroSPINTM Genomic DNA Purification kit. To pellet the cells, about 1.5 mL of cultures were transferred into microcentrifuge tubes and centrifuged at 12,000 rpm for 2 min. The supernatants were discarded, and the pellets were resuspended in 200 µL of Lysis Buffer LB-GD1. Once the suspension was homogenous, 20 µL of proteinase K was added, and it was incubated at 55 °C for 20 min. Then, 20 µL of RNase A was added, and the mixture was incubated at room temperature for 10 min. After adding 200 µL of ethanol and 200 µL of Lysis Buffer LB-GD2, the mixture was run through a PuroSPINTM MINI Spin Column with several washing steps.

Determination of quality of the extracted DNA

A nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.) was used to determine the DNA concentration. Purity was assessed by the 280/260 nm absorbance ratio. After connecting the nanodrop to a computer, cotton wool and 70% ethanol were used to clean the sensor. After using 1 µL of elution buffer as a blank, 1 µL of DNA samples were loaded onto the sensor. To avoid contamination, the sensor was cleaned in between the samples.

PCR amplification of the ITS region

The extracted DNA was used to amplify a

specific region called the internal transcribed spacer (ITS) using PCR. The primers used were ITS1 forward and ITS2 reverse. The PCR mixture contained DNA, primers, and a master mix. The PCR process involved heating and cooling the mix multiple times to amplify the DNA.

Gel electrophoresis

Following PCR, the DNA was separated and examined using gel electrophoresis. To make the DNA visible, a dye was added to a 2% agarose gel. A molecular weight marker and the PCR products were added to the gel. The DNA bands were visible under UV light after electric current was passed through the gel. By matching the DNA fragments to the marker, their sizes were determined. It was anticipated that fungal DNA would have bands between 200 and 400 bp.

Sanger sequencing of the fungal isolates

The study used Sanger sequencing to analyze soil samples, starting with purification of PCR products and setting up reactions using the BigDye™ Direct Cycle Sequencing Kit. The data were then analyzed to confirm and identify fungal species in the samples, providing a detailed workflow from DNA isolation to Sanger sequencing.

Phylogenetic tree analysis

Evolutionary history was inferred using the Neighbor-Joining method. Evolutionary distances were computed using the Jukes-Cantor method. The final dataset contained 668 places after five nucleotide sequences were examined. All ambiguous positions were removed for each pair of sequences. Evolutionary analysis was carried out using MEGA11.

Results and Discussion

Morphological and microscopic identification of fungi

The molecular characterization of the fungus linked to *Ficus* fruits was examined in this work. As seen in Fig. 2, two unidentified fungal species with the codes FF2 9-7 and FF2 5-8 were isolated and discovered to be connected to *Ficus* fruits. They both shown different physical characteristics. The colony of FF2 9-7 was initially white, but with age, it turned grey, dark grey, or olive-grey, and on the reverse side of the plates, it appeared yellowish. FF2 5-8 was yeast-like, mostly creamy or white colonies.

Molecular characterization of the fungi

The genomic DNA of isolated samples FF2 9-7 and FF2 5-8 was successfully isolated. The Nanodrop data showed the DNA concentrations of the isolates (Table 1 and Fig. 3). Nonetheless, to reduce sequencing expenses, isolates with the highest DNA contents were chosen. Fig. 4 displays

the results of the amplified PCR product obtained from the isolates. The ITS PCR procedure successfully amplified the desired ITS region in all the samples, with consistent amplification using FirePol Master Mix. Agarose gel electrophoresis checked product quality and size, while image processing tools evaluated band intensities for fungus. The FF2 9-7 and FF2 5-8 isolates' sequences were found to be 97.06% identical to *Dipodascus* sp strain F1 E06 and 80.83% identical to *Pestalotiopsis mangiferae* isolate MPK when compared to 100 sequences deposited in the National Center Biotechnology Information (NCBI) composite biological database. ITS markers have been used in many recent studies for molecular identification of fungi isolated from carrot (Javed and Javaid, 2021), lemon, tomato and seychelles pole bean (Khan and Javaid, 2022a, b, c).

Phylogenetic analysis

The phylogenetic tree created exposed the relationship between the isolates from this study and other fungal isolates in GenBank. The phylogenetic analysis showed that *Pestalotiopsis mangiferae* and *Dipodascus* sp. are closely connected to the fungal isolates obtained from the *F. sycomorus* fruit as presented in Fig. 5 and 6. Fungi have important functions in nature, yet their identification remains challenging. According to Pryce (2003), the traditional way of identifying fungi takes time, thus thorough isolation and characterization of fungi using molecular approaches is necessary. It may lack detail and be a lengthy process. In comparison to the traditional approach, the molecular method of identification promises to be more accurate, faster, and significant in the detection of plant diseases and pathogens. It also helps to identify other closely similar species. Similar to this, Aoki *et al.* (2003) argue that molecular research is important for identifying fungus at the species level, which is difficult to do merely by morphological characterization.

This study reports a new record of *P. mangiferae* and *Dipodascus* sp causing fruit rot disease in mulberry from Okochiri, Okrika Local Government Area, Rivers State, Nigeria. Molecular characterization identified these fungi as the causal agents of mulberry fruit rot in Rivers State, Nigeria. The findings of this research revealed the presence of the *Dipodascus* species as one of the fungal isolates associated with the fig-mulberry (*Ficus sycomorus*) fruit. This outcome aligns with previous report of Cannon and Kirk (2007) highlighting that members of the Dipodascaceae family are widely distributed and can be discovered as spoilage organisms in the food business or in decomposing plant tissue. The findings of Kurtzman *et al.* (2011) were in agreement with the fungal isolates of *Ficus* fruits. They reported in this investigation the presence of the several

species of *Dipodascus* occurring in the wound saps of trees or on fruits. *Dipodascus* sp. are members of the division of Ascomycota, Saccharomycetes class and the family Dipodiaceae is in the order Saccharomycetales. The fruit of the fig-mulberry is especially vulnerable to the *Dipodascus* species, which is known for its quick colonisation and prefers warm, humid conditions. This vulnerability emphasizes how crucial it is to use efficient post-harvest procedures in order to reduce fungal contamination (Sharma and Tripathi, 2017). Additionally, the second organism *P. mangiferae* is a member of the Ascomycota division, Sortariomycetes class, family Sporocadaceae and order Amphisphaeriales. Although it is a fungal plant pathogen attacking mangoes, it is reported to be a weak parasite able to infect young and old damaged leaves, wounded fruits and healthy fruits in touch with sick tissue. The results in this current study, are consistent with the findings of Perera *et al.* (2020). According to their findings, *Pestalotiopsis* sp was reported to be associated with dominant fungal genera on wild seeds and fruits.

The monetary influence of fungal contamination on fig-mulberry fruit is high. Fruits contaminated by fungal growth are often inappropriate for consumption and can result in large financial losses for farmers and suppliers (Ghafoor *et al.*, 2019). The presence of fungi not only disturbs the direct sale of the fruit but also interrupts the supply chain, affecting related industries such as food processing and export markets (Kalaiyarasi and Azhagu, 2021). *Dipodascus* species, in particular, can produce secondary metabolites that may cause health hazards if ingested, thereby intensifying the economic burden (Pitt and Hocking, 2009). The nutritional worth of *F. sycomorus* fruits further heightens the implications of fungal contamination. The fruit is high in vital minerals including calcium, vitamins A and C, and dietary fibre, all of which help the immune system, improve digestion, and fortify bones (Sena *et al.*, 2017). However, when fungi such as *Dipodascus* invade the fruit, they may break down these nutrients, reducing the fruit's general nutritional quality. certain fungi have been known to produce mycotoxins, which are harmful compounds that can cause adverse health effects in humans and animals (Bennett and Klich, 2003). The process by which *Dipodascus* species colonize and appear on fig and mulberry fruit is complex. Fungal penetration into the host happens through natural holes, wounds, or undamaged surfaces (Chethana *et al.*, 2021), where they use enzymes to break down the cellular structure and initiate attack. This enzyme activity can degrade the fruit's texture and flavour, rendering it unpleasant and unsuitable for both direct ingestion and processing (Al-Hindi *et al.*, 2011). Effective preventative procedures, such as careful handling, regulated storage, and the application of antifungal medications, are required to reduce the spread and

effect of fungal contamination (Moss, 2008).

Conclusion

This study successfully identified *Pestalotiopsis mangiferae* and *Dipodascus* sp. as the primary fungal pathogens responsible for post-harvest rot in *Ficus sycomorus* fruits in Rivers State, Nigeria. The combination of morphological and molecular characterization using ITS markers confirmed the pathogenic roles of these fungi, with sequence similarities of 80.83% and 97.06%, respectively. Given the economic and medicinal importance of *F. sycomorus*, these findings are critical for developing targeted disease management strategies to reduce post-harvest losses. The study also highlights the need for further research into

potential mycotoxin risks and strategies to mitigate fungal deterioration, ultimately contributing to improved food security and public health outcomes.

Contribution of authors

JOT Conceived the idea and wrote the first draft of manuscript; MCF and COO performed the experiments, analyzed and interpreted the data; CGIN supervised the study, handled the literature survey, funded part of reaserch and performed final editing of the manuscript.

Conflict of interests

Authors declare no conflict of interest.



Fig. 1: *Ficus sycomorus* fruits.

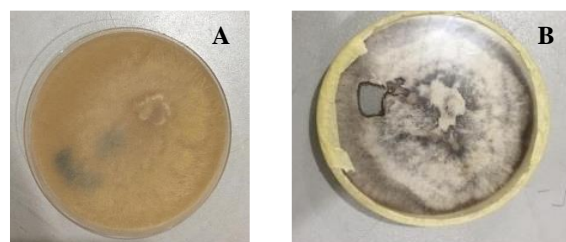


Fig. 2: Pure cultures of fungal sample FF2 5-8 (A) and FF2 9-7 (B), isolated from fig fruits.

Table 1: Table of DNA Concentration.

Sample Name	DNA Conc. (ng μL^{-1})	A260	A280	Purity index (260/280)	Factor
FF2 9-7	14	0.280	0.137	2.05	50
FF2 5-8	28.7	0.574	0.296	1.94	50

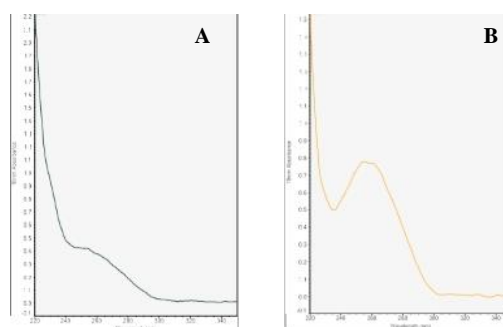


Fig. 3: Graph showing concentration of DNA extracted from fungal isolate of FF2 9-7 (A) and FF2 5-8 (B).

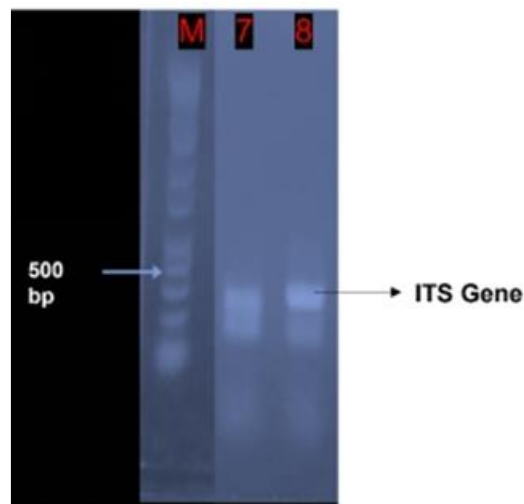


Fig. 4: Amplified PCR product generated from FF2 9-7 and FF2 5-8 isolates.

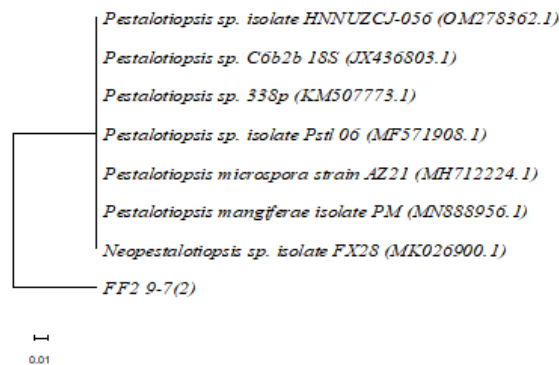


Fig. 5: Phylogenetic tree of isolate FF2 9-7

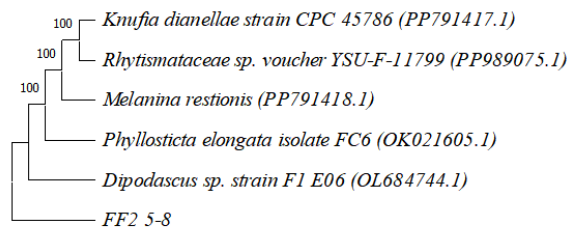


Fig. 6: Phylogenetic tree of isolate FF2 5-8.

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