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Identification of *Lasiodiplodia pseudotheobromae* Causing Leaf Blight Disease of *Thaumatococcus daniellii* (Benn.) Benth (Sweet Prayer Plant)

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Authors' contributions

This work was carried out in collaboration among all authors. Author CGIN designed the study. Author JA performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author CGIN managed the analyses of the study, the literature searches and edited the article for submission. All authors read and approved the final manuscript.

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ABSTRACT

This study is aimed at isolating and identifying the common fungal pathogens causing leaf blight disease of *Thaumatococcus daniellii* known as the sweet prayer plant, using the molecular technique. It is a highly nutritional plant, used as laxatives, venom antidote, sedative and in the treatment of diabetes mellitus. Despite its global popularity owing to the usefulness of the leaves in food wrapping and packaging, it has been observed that the plants suffer severe leaf blight disease caused by fungal pathogens. Samples of leaves showing disease symptoms were collected from the Umuakali community in Omuma Local Government Area of Rivers State between June 2021 and October 2022. Fungal isolates were collected from leaves and morphologically identified. The

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DNA of the most common fungal isolate, SPP-01, was molecularly characterized using Internal Transcribed Spacer 1 (ITS-1) molecular markers. Sequences obtained were subjected to BLAST search in the GenBank database. The morphological results indicated that the SPP- 01 isolate was a *Lasiodiplodia* species. The molecular weight of the DNA of the isolate was over 550 Base Pairs. Based on sequence similarity, the DNA sequence of the isolate was 99% identical to *Lasiodiplodia pseudotheobromae*. A pathogenicity test of the isolated pathogen was carried out. Therefore, these findings showed that *L. pseudotheobromae* is the causal fungal pathogen of leaf blight disease of the sweet prayer plant. It is expected that this finding will promote the acquaintance of the fungal species associated with the sweet prayer plant and provide information for developing an effective disease management strategy for mitigating the losses caused by *L. pseudotheobromae* and also provide the basis for further study of potential mycotoxin effect of consuming disease leaves. To the best of our knowledge, this is the first report of molecular characterization of *L. pseudotheobromae* infesting sweet prayer plant in Rivers State.

Keywords: Thaumatococcus daniellii; Lasiodiplodia pseudotheobromae; pathogenicity; RBCL marker.

1. INTRODUCTION

"The sweet prayer plant (*Thaumatococcus daniellii*) is a multipurpose perennial [1] that grows in hot, humid tropical rainforests and coastal zone of West Africa". "It is also known to grow over large areas of East and Central Africa" [2,3]. It is a common plant found in Ghana and Nigeria whose leaves are used by the residents to wrap and cook moimoi (bean pudding) [4], especially in the Southern, Eastern, and most parts of the Western part of Nigeria. Similarly, Swift et al. [5], stated that "it is used to wrap vegetables, fish, and cola nuts and as a source of supplementary fodder for livestock in many African households. It is an herbaceous rhizomatous perennial shrub that grows up to 3-3.5 meters in height. It has ovate, large, papery, and elliptical leaves that are up to 60cm long and 40cm wide and sprout from each node of the rhizome singly".

T. daniellii, has gained global popularity and interest owing to the use of its leaves in food wrapping and packaging. Apart from the usefulness of the leaves, it is also harvested for their fruits, where a protein-based sweetener called thaumatin is extracted from their rinds [3] and used by the food and confectionary industry for substituting synthetic sweeteners. According to Giwa, [4], research has shown that the plant is a natural sweetener which they claim is about 3,000 times sweeter than sugar. Sweeteners excellent for diabetics can be extracted from the fruits of this plant. It contributes to the economy of the rural people [6,4]. There is an unexploited potential for *T. daniellii* fruits that improves the livelihoods of rural people in West and Central Africa [7] in terms of employment [3]. The leaves of this plant, have good flavor and preservative effects which is the reason why it is a choice for food wrapping [8]. This plant is used to treat pulmonary (lung) challenges, and leaf sap is useful as an antidote for venoms, bites, and stings. It is used as a sedative and as a treatment for insanity. Prevents and reverses oxidative damage in the liver and the kidneys' jaundice or sickle cell crisis.

There has been an increase in the demand for *Thaumatococcus daniellii* Benn (Benth.) leaves as food wrappers in indigenous fast food and food processing industries over the use of synthetic ones (such as plastics and nylons). Furthermore, it is used in making thatch root, waiving baskets, mats, bags, hats, hand fans, and also as a taste modifier [9,10]. The cultivation of this plant is threatened by some phytopathogenic organisms that affect health negatively. An example of phytopathogenic organisms is fungi. "Fungal disease can damage plants and crops, causing major losses in agricultural activities and food production" [11]. Plant diseases have been reported to play a direct role in destroying natural resources in agriculture. This study aimed at isolating and identifying the fungal pathogens associated with severe leaf blight disease of *Thaumatococcus daniellii* using the molecular method.

2. MATERIALS AND METHODS

2.1 Source of Plant Material

Samples of leaves showing diseased symptoms were collected from Umuakali community in Omuma Local Government Area of Rivers State.

2.2 Isolation of Fungi from *Thaumatococcus danielli* using Blotter Method

Fungal pathogens associated with sweet prayer leaves were isolated using a standard blotter method modified by Ikechi-Nwogu et al. [12]. The diseased leaves were cut from the lesion area, disinfected by dipping into 70% ethanol, rinsed twice toro thrice with sterile distilled water, and each piece of leaf, was transferred into Petri dishes lined with 3 layers of the sterilized filter paper and incubated for 7 days at a temperature of 25 $\pm$ 2^{o}C at the Pathology/Mycology laboratory of the Department of Plant Science, University of Port Harcourt, Rivers State, Nigeria. At the end of the seven days, fungal colonies found growing on the leaves, were transferred into 9cm Petri-dishes containing Potatoes Dextrose Agar (PDA) and incubated for 7 days at a temperature of 25 $\pm$ 2^{o}C and the most common fungal isolate was coded (SPP-01).

2.3 Morphological and Microscopic Characterization and Identification

The fungal mycelium of isolate SPP-01 was cultured on Potato Dextrose Agar medium at room temperature for one week. Identification of the SPP-01 isolate was done by visual observation of the mycelium and pictorial comparison using Snowdon [13]. The isolate was subjected to microscopic analysis by staining wet mounts from the pure culture with lactophenol blue for easy characterization and identification under a monocular microscope at X10.

2.4 Molecular Characterization Using the Internal Transcribed Spacer (ITS) Marker and Identification

The DNA extraction and quantification were carried out with modifications in the Molecular Laboratory of the Department of Pharmacy, Niger Delta University, Bayelsa State, Nigeria. The Genomic DNA of the isolate was extracted following the protocol of a Zymo Research Fungal/Bacterial DNA MiniPrep Extraction Kit as described by the manufacturer. The extracted genomic DNA was then quantified using a Nanodrop 1000c spectrophotometer. The DNA samples were shipped to Inqaba Biotechnological, Pretoria South Africa for amplification and sequencing. For amplification, the Internal Transcribed Spacer 1F (ITS1F) 5'-CTTGGTCATTTAGAGGAAGTAA-3' and Internal Transcribed Spacer 4 (ITS4) 5'-TCCTCCGCTTATTGATATGC-3' primers were used to amplify the ITS regions of the isolates on an ABI 9700 Applied Biosystems Thermal Cycler at a final volume of 30 µl for 35 cycles. Sequencing was done using the Big Dye Terminator kit on a 3510 ABI sequencer at a final volume of 10 µl for 32 cycles. Obtained sequences were edited using the bioinformatics algorithm trace edit. Similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool for Nucleotide (BLASTIN). These sequences were aligned using MAFFT (for multiple alignments using fast Fourier transform). The evolutionary history was inferred using the Neighbor-Joining method in Molecular Evolutionary Genetics Analysis (MEGA) version 6.0.

3. RESULTS AND DISCUSSION

3.1 Isolation, Morphological and Microscopic Identification of Fungi Associated with *Thaumatococcus danielli*

The result of the fungal isolation of fungal pathogens from diseased leaves is presented in plate 1 below. The fungal colonies on PDA were initially white with feathery aerial mycelia (plate 1a). The fungal colonies then became grey (plate 1b) while the reverse side became grey to black (plate 1c). The isolated fungus was initially identified as belonging to the genus *Lasidioplodia.*

3.2 Molecular Characterization Using the Internal Transcribed Spacer (ITS) Marker and Identification

The genomic DNA of the isolates SPP-01 of the sweet prayer plant was successfully extracted. The NanoDrop result showed that the concentration of the DNA of the isolates was 15.1 ng/µl. The result of the amplified PCR product generated from the SPP-01 isolate is shown in Plate 2 below. The amplified DNA showed a band on the gel when observed under UV light. From the result, the ladder used indicated that the SPP-01 isolate sequence had over 550 base pairs.

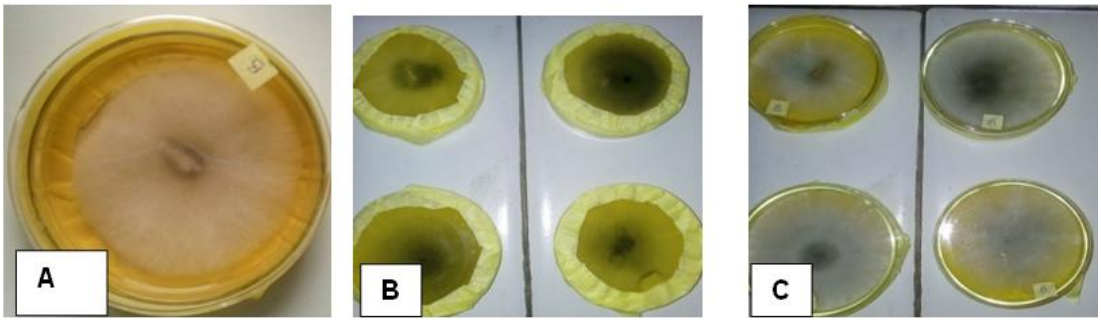

Plate 1. Morphological characteristics of *Lasiodiplodia* sp. recovered from diseased leaves of *Thaumatococcus daniellii*. (A) Initial appearance of Fungal colonies (B) Upper view of the colony appearance (C) Reverse view of colony appearance

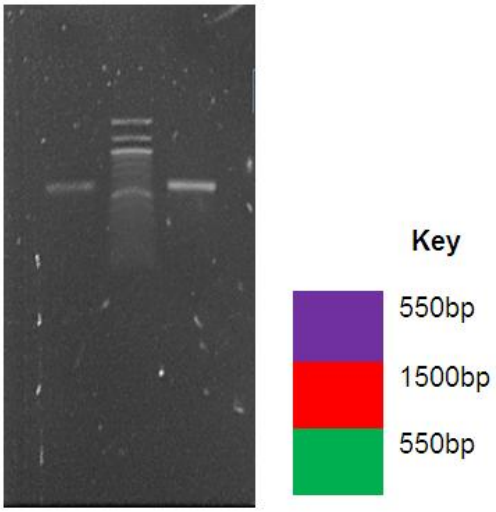

Plate 2. Amplified PCR product generated from SPP-01 isolate

The result of the SPP-01 isolate sequence alignment is presented in Plate 2 below. The result indicated that the SPP-01 isolate sequence aligned with 100 sequences deposited in the composite biological database of National Center Biotechnology Information (NCBI) was 99% identical to *Lasiodiplodia pseudotheobromae.*

"The molecular techniques used in the identification of fungi in this study led to the successful characterization of a fungus isolated with leaf blight diseased of the sweet prayer plant. The fungus obtained from this study belongs to the division, class Dothideomycetes, order Botryosphaeriales, and family Botryospheriaceae. Lasiodiplodia species have been recorded from many hosts, manifesting as pathogens" [14] "*Lasiodiplodia pseudotheobromae* was first described from Gmelina arborea in Costa Rica" [15]. "It was later isolated from different parts of many host plants' necrotic shoots and branches of Mangifera indica [16] and rot stems of *Ormosia pinnata"* [17]. Based on morphology and analysis of the internal transcribed spacer (ITS), *Lasiodiplodia pseudotheobromae* was also identified in Thailand as the causal fungus of fruit rot disease of post-harvest longan - *Dimocarpus longan* (Pipattanapuckdee et al. 2019). It has also been reported by Correia et al. [18] *"*as a grapevine trunk pathogen in Brazil and is mostly found in Africa, Europe, and Latin America" (Adetunji and Oloke, 2013). "In Thailand, *Lasiodiplodia pseudotheobromae* has been reported also to cause canker, decline, dieback, stem end rot, and fruit rot on a wide range of plants" (Farungsang et al. 1992; Trakunyingcharoen et al. 2013). According to Rosado et al., 2016, "Lasiodiplodia pseudotheobromae is commonly found as endophytes and pathogens of various plants in tropical and subtropical regions". "They are generally regarded as opportunistic pathogens as they cause diseases when the host

plant is exposed to stress or favourable conditions for disease development" [19].

In this study, we provide a new record of *L. pseudotheobromae* causing leaf blight diseased leaves of the sweet prayer plant from Umuakali community in Omuma Local Government Area of Rivers State, Nigeria based on morphology and analysis of the internal transcribed spacer (ITS). The result is in agreement with the study conducted by Aroge et al. (2019). In their study, they stated that the sweet prayer plant is affected by severe leaf blight disease. The harm of *L. pseudotheobromae* to sweet prayer plants during the storage period has not been evaluated although the fungus can artificially infect sweet prayer plants.

4. CONCLUSION

In this study, we provide a new record of *L. pseudotheobromae* causing leaf blight disease of sweet prayer plant from Rivers State, based on identification using phylogeny and morphology.
More researches are needed to clarify the primary inocula and the inoculum source of the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hasan MA, Norrizah JS, Ali HJ, Amjad AM, Abdulmutalib AA. Overview of Thaumatococcus Daniellii Plant, History, Uses, Benefits, and Characterization. J. of Asian Sci. Res. 2022;12(2):80-90.
2. Yeboah SO, Hilger TH, Kroschel J. Thaumatococcus daniellii: A Natural Sweetener from the Rain Forest Zone in West Africa with Potential for Income Generation in Small Scale Farming, Institute of Plant Production and Agroecology of the Tropics and Subtropics, Hohenheim University. 2003;1:1.
3. Ekpe KE, Ottou R. Benefiting from conservation: how the collection of *Thaumatococcus daniellii* fruits in Ghana is emerging as a forest industry, Silvanet. 2006;19(1):2–4.
4. Giwa A. Sweetener from *Thaumatococcus daniellii*. 2013 Accessed 29 November 2012.
Available: Sweetener from
Thaumatococcus daniellii available at:
Vanguard News (vanguardngr.com)
accessed on 19-11-2022.
5. Swift KAD, Pearce S, Roth H, Out of Africa: the chemistry and flavour properties of the protein thaumatin, in Advances in Flavours and Fragrances, K. A. D. Swift, Ed., Royal Society of Chemistry. 2002; 178–193.
6. Arowosoge OGE, Popoola L. Economic analysis of *Thaumatococcus danielli* (Benn.) Benth. (Miraculous berry) in Ekiti State, Nigeria, Journal of Food, Agriculture and Environment. 2006;4(1):264–269.
7. Wojciech S, Oppong S, Hall JB, Sinclair FL. Implications of local knowledge of the ecology of a wild super sweetener for its domestication and commercialization in West and Central Africa, Economic Botany. 2005;59 (3):231–243.
8. Aroge T, Akanmu AO, Abiala MA, Odebode JA. Pathogenicity and in vitro extracts inhibition of fungi causing severe leaf blight in *Thaumatoccocus danielli* (Benn.) Benth. Archives of Phytopathology and Plant Protection. 2019; 52:1-2:54-70.
DOI: 10.1080/03235408.2019.1572055.
9. Adu O, Adeyemo A, Falua O, Fajana O, Ogunrinola O, Saibu M, Elemo B. The Effect of *Thaumatococcus danielli* Leaf Extracts on Immunological and Oxidative Stress Markers in Rat. Asian J. of Biochem., Gen. and Molecular Bio. 2021;7(4):6-14.
Available:
10.9734/AJBGMB/2021/v7i430179.
10. Olabanji SO, Osinkolu GA, Pelemo DA, Obiajunwa EI, Oladele AT. PIXE analysis of Thaumatococcus danielli in Osun State of Nigeria. Nucl Instrum Methods Phys Res B. 2014; 318:182–186.
11. Savary S, Ficke A, Aubertot JN, Hollier C. Crop losses due to diseases and their implications for global food production losses and food security. Food Secur. 2012;4:519–537.
DOI: 10.1007/s00203-017-1426-6.

12. Ikechi-Nwogu CG, Amos J. Characterization of Post-Harvest Fungal of (*Solanum lycopersicum* L.) fruits Tomato Niger J. Mycol. 2020;12(1):54-62.
13. Snowdon AL. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables: General Introduction and Fruits. Wolfe Scientific. 1990;1-302.
14. Abdollahzadeh J, Javadi A, Goltapeh EM, Zare R, Phillips AJL. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. Persoonia. 2010; 25:1–10.
15. Alves A, Crous PW, Correia A, Phillips AJL. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Divers. 2008;28:1–13.
16. Kwon JH, Choi O, Kang B, Lee Y, Park J, Kang DW, Han I, Kim J. Identification of *Lasiodiplodia pseudotheobromae* causing mango dieback in Korea. *Can. J. Plant Pathol.* 2017; *39:241*–245.
17. Li FF, Liu JQ, Chen QL. *Botryosphaeriaceae* from Eucalyptus plantations and adjacent plants in China. Persoonia. 2018;40:63–95
18. Correia KC, CÂMara MPS, Barbosa MAG, Sales R, AgustÍ-Brisach C, Gramaje D, LeÓN M, GarcÍA-JimÉNez J, Abad-Campos P, Armengol J, et al. Fungal trunk pathogens associated with table grape decline in North-eastern Brazil. Phytopathol. Mediterr. 2013;52:380–387.
19. Slippers AD, Wingfield B, Roux MJ, Botryosphaeriaceae Associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. Mycol. Prog. 2010;9:101–123.

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