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Etiological agents of crown rot disease in Cavendish bananas in Colombia

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ABSTRACT

Crown rot is a major postharvest disease affecting Colombian bananas exported to Europe. Its etiology involves a complex of fungi influenced by geography and climate, yet the causal agents in Urabá, Colombia's main Cavendish banana export region, remain unclear. We collected and characterized 95 fungal isolates from diseased crowns using classical taxonomy and whole genome sequencing. The most frequent genus was *Fusarium* (65.3%), followed by *Clonostachys* (14.7%), *Colletotrichum* (11.6%), and others at lower frequencies. Only *Fusarium musae* and *Colletotrichum* sp. reproduced crown rot symptoms and fulfilled Koch's postulates. *Colletotrichum* sp. caused more severe infections, while *F. musae* showed slower growth. This is the first confirmed report of banana crown rot etiology in Colombia.

Keywords: Postharvest diseases, *Fusarium*, *Colletotrichum*, Cavendish, Banana

RESUMEN

La pudrición de corona es una de las principales enfermedades postcosecha que afecta los bananos exportados desde Colombia hacia Europa. Su etiología involucra un complejo de hongos influenciado por condiciones geográficas y climáticas; sin embargo, los agentes causales en Urabá, principal zona exportadora de banano Cavendish, no se conocen con precisión. En este estudio se colectaron y caracterizaron 95 aislamientos fúngicos mediante taxonomía clásica y secuenciación genómica. El género más frecuente fue *Fusarium* (65,3 %), seguido de *Clonostachy*s (14,7 %), *Colletotrichum* (11,6 %) y otros en menor proporción. Solo *Fusarium musae* y *Colletotrichum* sp. reprodujeron los síntomas y cumplieron los postulados de Koch. Este constituye el primer reporte confirmado de los agentes causales de la pudrición de corona en Colombia.

Palabras clave: Postcosecha, *Fusarium*, *Colletotrichum*, Cavendish, Banano

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INTRODUCTION

Bananas are one of Colombia's most important export products, ranking third after coffee and flowers, which makes them vital to the country's economy (AUGURA 2025). Currently, 52,753 hectares are dedicated to banana cultivation, with 32,883 hectares located in the Urabá region of Antioquia, the country's primary banana-exporting area. In 2024, the average productivity was 2,073 boxes per hectare, resulting in a total value of $1026,4 million, with 109,9 million boxes exported (AUGURA 2025). The criteria for defining banana quality include appearance, aroma, taste, safety, and shelf life. Consequently, failing to meet these standards can result in market restrictions, a decrease in overall product volume, or a drop-in value. As a result, fruit losses can happen during harvesting, packing, transportation, storage, and marketing, with losses for bananas ranging from 20% to 80% of production (Yahia *et al*., 2019). Most quality defects encountered by fruits exported to European and American markets during the winter season arise from post-harvest diseases, particularly crown rot. This condition is caused by a complex of fungi that not only impacts the fruit's appearance and quality but also shortens the shelf life of the bunches. In severe cases, the fingers of the fruit may even detach from the crown (De Melo *et al*., 2016). However, the population of microorganisms associated with crown rot varies according to the climatic conditions and geographical location of the farm (Ewane *et al*., 2013). The objective of this study was to identify and characterize the causal agents of crown rot in Cavendish bananas (AAA) in the Urabá region of Colombia. This marks the first report of the microorganisms responsible for this disease in the country.

MATHERIALS AND METHODS

Sampling, pathogen isolation, and morphological characterization

Sample collection was conducted in four commercial Cavendish banana plantations (*Musa* AAA) of the Urabá subregion in the department of Antioquia, Colombia. A total of 40 clusters of 11-week-old Cavendish bananas were sampled at two locations in the packinghouse: fruit reception area and the wash tank. Afterward, the fruit was placed in a humid chamber at 25 ºC with 12 hours of darkness to promote the sporulation of microorganisms in the crowns. The fungi were isolated from 1 cm^2 segments of crowns, following the disinfection methodology described by Abd-Alla *et al*. (2014). The isolates were incubated at a temperature of 25 ºC for six days. After this period, colonies with similar morphology were selected and purified on PDA with Streptomycin. The taxonomic characterization of the isolates was conducted using classical taxonomic methods, achieving genus-level identification where possible, in accordance with the approaches described by Seifert *et al*. (2011).

Pathogenicity tests

At 8 days of age, isolates were collected, and sterile distilled water was added. Scraping was then performed using a Drigalski we adjusted the concentration of each strain to 5 x 10^5 conidia/ml. Finally, these two individual suspensions were combined to create a final inoculum with a concentration of 1 x 10^6 conidia/ml. Next, 200 µl of this suspension was transferred using a micropipette and inoculated onto the two lateral faces and the top of the crowns for each of the isolates. The fruit was then placed in a humidity chamber within polyethylene bags to create optimal humidity and temperature conditions for fungal growth. The banana clusters were maintained at a temperature of 16 ºC with 12 hours of light, and disease symptoms were monitored every 72 hours. The development of symptoms was observed and recorded up to 8 days after inoculation to monitor lesion development. Once the first symptoms of crown rot appeared, we re-isolated the inoculated clusters by taking 1 cm x 1 cm segments. These segments were then placed on Petri dishes containing PDA for further analysis, to fulfill *Koch's* third and fourth postulates.

DNA extraction and data sequencing

Isolation of total genomic DNA from fungal cultures was performed with the Qiagen DNeasy PowerLyzer PowerSoil Kit, following the manufacturer's recommendations. DNA integrity was evaluated by agarose gel electrophoresis, and its concentration was quantified by light absorption at 260nm using the NanoDropTM 2000-Thermo ScientificTM and the Picogreen fluorescent method. The NGS sequencing experiment was performed on an Illumina NovaSeq instrument reading 150 paired-end reads. Raw reads were filtered using the CUTADAPT software v2.10, Genomic assembly was performed with SPADES assembler v3.14.1(Bankevich *et al*., 2012), using default parameters.

Phylogenetic Analysis and Comparative Visualization

For robust phylogenetic reconstruction, sets of single-copy orthologous proteins were identified and extracted from the *de novo* assembled genomes of *Fusarium musae* (CEN02) and *Colletotrichum* sp. (CEN01). High-quality multiple sequence alignments were generated using MAFFT v7.490 (Katoh and Standley, 2013), incorporating homologous protein sequences retrieved from publicly available fungal genome databases for both genera to maximize taxon sampling and improve phylogenetic resolution. Phylogenetic inference was performed with IQ-TREE2 v2.1.3 (Nguyen *et al*., 2015) using the maximum-likelihood method and 5,000 ultrafast bootstrap (UFB) replicates to ensure robust branch support.

RESULTS AND DISCUSSION

Isolation and morphological identification

The genera isolated from Cavendish banana crowns exhibiting characteristic disease symptoms were identified with the following frequencies: *Fusarium* (65.3%) was the most prevalent, followed by *Clonostachys* (14.7%), *Colletotrichum* (11.6%), *Curvularia* (3.2%), *Penicillium* (3.2%), *Pestalotia* (1.1%), and a fungus from the order Mucorales (1.1%). *Fusarium* was the most frequently isolated genus at both locations, accounting for 42.86% of samples from the barcadilla and 17.14% from the wash tank. *Clonostachys* was most found in the barcadilla location, with a frequency of 8.57%. Isolates of *Colletotrichum* were noted in 5.71% of samples taken from the wash tank. Furthermore, *Curvularia* and *Penicillium* were exclusively found in the barcadilla, each with a frequency of 2.86%. *Pestalotia* was solely isolated from the wash tank, at a frequency of 0.95%.

Pathogenicity tests

The study found that isolates from the genera *Clonostachys*, *Penicillium*, and Mucorales were not pathogenic to the crowns of Cavendish bananas. In contrast, *Colletotrichum* sp. and *Fusarium musae* were identified as the most pathogenic (Figure 1). *Colletotrichum* sp. and *Fusarium musae* were able to reproduce the typical symptoms of crown rot. *Colletotrichum* sp. had shorter incubation periods (4 to 5 days) and latency periods (4 to 7 days), as well as larger lesions, measuring 11 to 14.6 cm. In comparison, *Fusarium musae* exhibited longer incubation periods (4 to 12 days) and latency periods (9 to 15 days), with smaller lesions measuring 1 to 11 cm. Both *Fusarium musae* and *Colletotrichum* sp. isolates satisfied *Koch's* postulates.

Phylogenetic Placement and Genomic Comparison of the *Colletotrichum* Isolate Associated with Crown Rot in Cavendish Bananas

Phylogenomic analyses based on 2,620 single-copy orthologous proteins robustly positioned the Colombian isolate *Colletotrichum sp*. CEN01 within the C. *gloeosporioides* species complex (CGSC) (Figure 2). The isolate clustered with *C. viniferum, C. asianum*, and other members of the *C. gloeosporioides* species complex (CGSC), forming a well-defined monophyletic clade within the complex. Despite this clear placement, CEN01 could not be definitively assigned to any currently described *Colletotrichum* species, suggesting it may represent a novel and as-yet uncharacterized lineage currently absent from publicly available genomic databases.

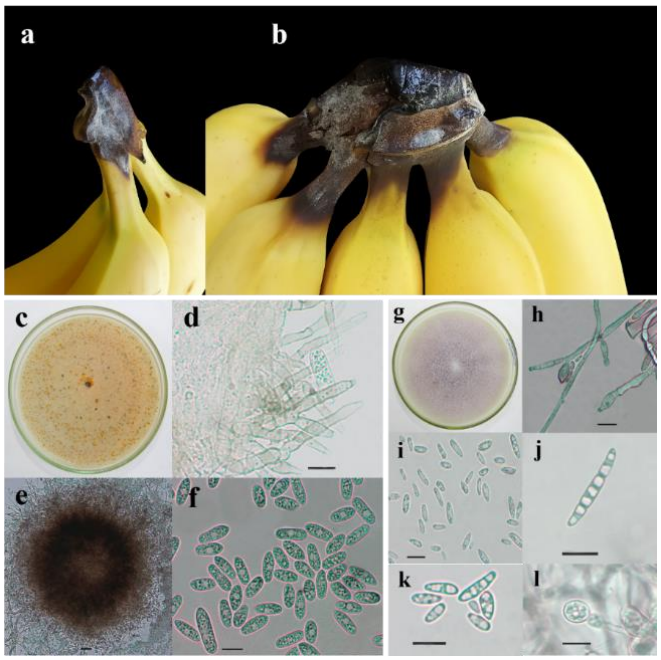


Figure 1. Crown rot symptoms in banana fruit and morphological characteristics of *Colletotrichum* sp., and *Fusarium musae*; (**a** and **b**) Crowns diseased with a superficial mycelium; (**c** - **f**) *Colletotrichum* sp.; (**c**) Colony on PDA after 8 days of incubation; (**d**) Setae; (**e**) Acervulus; (**f**) Conidia; (**g** - **l**) *Fusarium musae*; (**g**) Colony on PDA after 8 days of incubation; (**h**) Conidiophores with conidia; (**i**) Microconidia; (**j** and **k**) Macroconidia; (**l**) Chlamydospores. Bar = 10 µm in c, d, f, g, h, i, j, k, and l and bar = 50 µm in e.

Phylogenetic placement of *Fusarium musae* isolate associated with crown rot in Cavendish bananas

A phylogenomic analysis based on 220 single-copy orthologous proteins placed the Colombian isolate *Fusarium musae* CEN02 within the *F. musae* clade, supported by maximal branch support values (Figure 3). The isolate clustered tightly with the reference genome of *F. musae* (GCF_019915245), confirming its identity as this banana-associated pathogen. The strong branch support values underscore the robustness of this placement and highlight the genetic distinctiveness of *F. musae* relative to closely related taxa, such as *F. verticillioides and F. napiforme*. These findings validate *F. musae* CEN02 as a causal agent of crown rot disease in Cavendish bananas in Colombia and provide a genomic reference for comparative studies on pathogenicity and host specificity within the *Fusarium fujikuroi* species complex (FFSC).

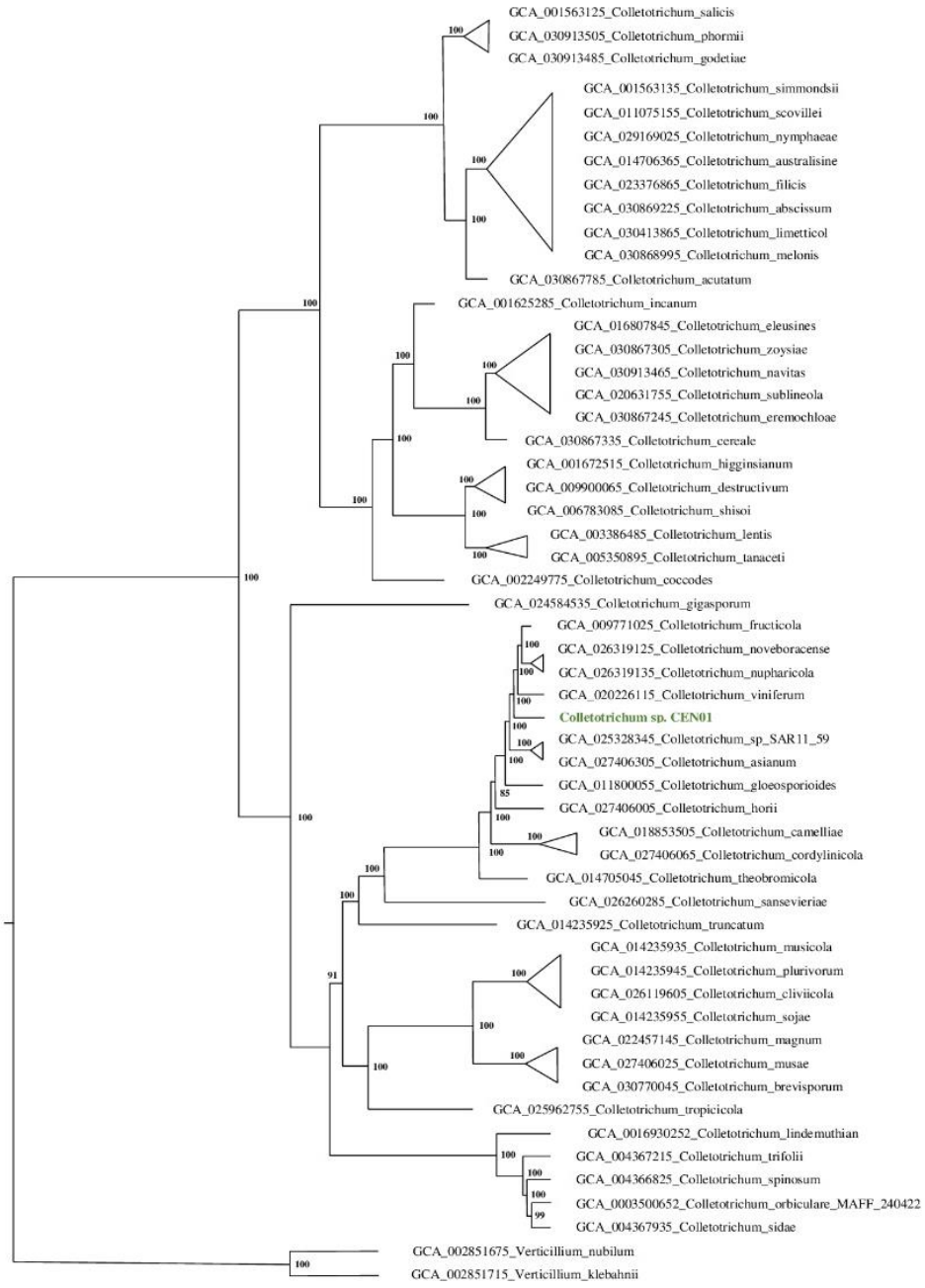


Figure 2. Phylogenetic tree showing relationships of the isolate *Colletotrichum* sp. CEN01 within the *C. gloeosporioides* species complex (CGSC). High-quality multiple sequence alignments were generated using MAFFT v7.490. Phylogenetic inference was performed with IQ-TREE2 v2.1.3 using the maximum-likelihood method and 5,000 ultrafast bootstrap (UFB) replicates to ensure robust branch support.

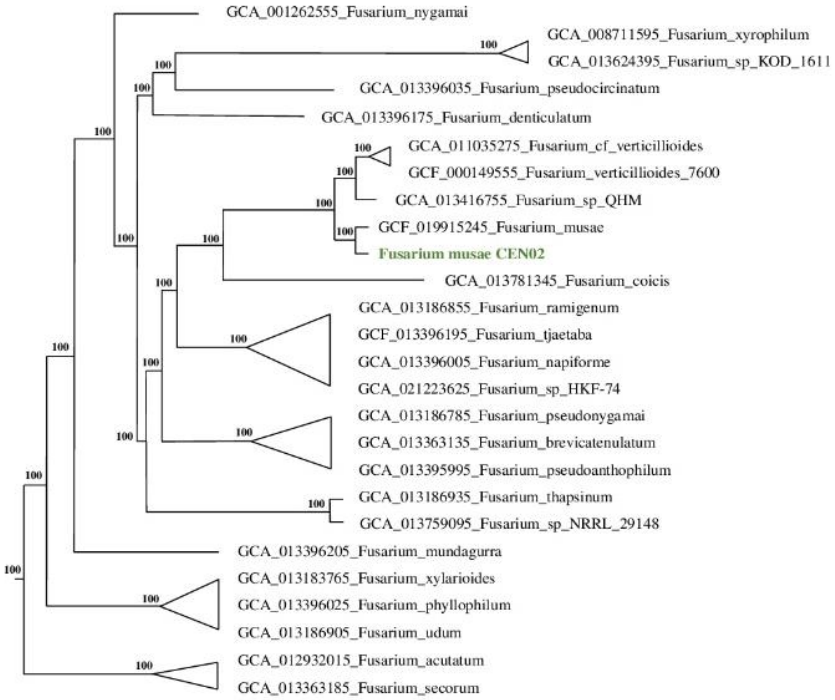


Figure 3. Phylogenetic tree showing relationships of the isolate *Fusarium musae* CEN02 within the *F. musae* clade, supported by maximal branch support values. High-quality multiple sequence alignments were generated using MAFFT v7.490. Phylogenetic inference was performed with IQ-TREE2 v2.1.3 using the maximum-likelihood method and 5,000 ultrafast bootstrap (UFB) replicates to ensure robust branch support.

CONCLUSIONS

This study provides the first genomic confirmation of *Fusarium musae* as a causal agent of crown rot disease in Colombia's Uraba region**,** a strategic center for Cavendish banana production and export. While *F. musae* has previously been reported from Latin America, the Canary Islands, and the Philippines (Kamel *et al*., 2016), these identifications were largely based on morphological traits or limited multilocus sequencing approaches. Our comprehensive phylogenomic analyses unequivocally place isolate CEN02 within the *F. musae* clade, extending the known distribution of this pathogen and emphasizing its epidemiological significance in Latin America. The availability of a high-quality genome assembly for CEN02 provides an essential resource for comparative genomics, population-level studies, and molecular diagnostics, strengthening postharvest disease management strategies and safeguarding the phytosanitary status of Colombian banana exports.

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