ISOLATION, PURE CULTURE ACQUISITION, AND MICROSPOPIC IDENTIFICATION OF THE PHYTOPATHOGENIC FUNGUS *ALTERNARIA* FROM INFECTED PLANT TISSUES

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Abstract.

Phytopathogenic fungi of the genus *Alternaria* are major causal agents of various plant diseases, causing substantial pre- and post-harvest economic losses globally. This study focuses on the systematic isolation, pure culture establishment, and morphological identification of *Alternaria* strains from symptomatic plant tissues. Infected leaves exhibiting typical concentric target-board necrotic spots were surface-sterilized using sodium hypochlorite, rinsed in sterile distilled water, and inoculated onto Potato Dextrose Agar (PDA). Following incubation at 25±2 °C emerging fungal colonies were purified using the single-spore and hyphal tip isolation techniques. Microscopic examination of the pure cultures under a light microscope revealed typical dark-pigmented (dematiaceous), septate mycelia and characteristic obclavate, muriform conidia bearing longitudinal and transverse septa, often arranged in chains. These distinct morphological diagnostic traits reliably confirmed the isolated strain as belonging to the genus *Alternaria*. The obtained pure cultures serve as a valuable reference collection for subsequent physiological, molecular, and pathological assays.

Keywords: *Alternaria*, phytopathogen, isolation, pure culture, microscopic identification, morphology.

Introduction.

The genus *Alternaria* comprises a widespread group of anamorphic, ubiquitous

fungi encompassing saprophytic, endophytic, and severe phytopathogenic species. Many *Alternaria* species are notorious plant pathogens responsible for early blight, leaf spots, fruit rots, and blights on a broad range of economically important agricultural and horticultural crops, including solanaceous plants, brassicas, and cereals. Beyond causing massive agricultural yield losses, several strains are capable of producing hazardous mycotoxins that threaten food safety.

Accurate identification and timely isolation of plant pathogens from infected hosts are foundational prerequisites for effective plant disease management, epidemiological tracking, and resistance breeding programs. Although molecular diagnostics such as PCR and sequencing provide high specificity, classical microbiological isolation paired with precise light microscopy remains the indispensable first step for recovering viable reference cultures and evaluating primary morphological phenotypes. Therefore, this study aimed to optimize the protocol for isolating, purifying, and morphologically characterizing *Alternaria* isolates from symptomatic plant tissues under laboratory conditions.

Materials and methods

Sample Collection and Surface Sterilization: Symptomatic plant organs (leaves and fruits showing characteristic brown, concentric necrotic lesions) were collected from field crops. The samples were washed thoroughly under running tap water to remove surface dust. Under aseptic conditions in a laminar flow hood, small tissue sections (5×5 mm) encompassing the boundary between healthy and diseased tissues were excised. The fragments were surface-sterilized by immersion in 1.0% sodium hypochlorite (NaOCl) solution for 1-2 minutes, followed by rinsing three times in sterile distilled water to eliminate residual disinfectant.

Inoculation and Cultivation: The surface-sterilized tissue pieces were blot-dried on sterile filter paper and aseptically transferred onto Potato Dextrose Agar (PDA) Petri dishes supplemented with streptomycin (50 mg/L) to suppress bacterial

contamination. The inoculated plates were incubated in an incubator in the dark at 25±2°C for 4 to 7 days.

Pure Culture Acquisition: To obtain axenic (pure) cultures, mycelial tips emerging from the margins of the plant tissues were aseptically transferred onto fresh PDA plates using a sterile inoculating needle. Subculturing was repeated until homogeneous, stable pure colonies were established.

Microscopic Examination: Morphological characterization was performed using a compound light microscope. Slides were prepared using lactophenol cotton blue stain. Micromorphological features-including mycelial structure, color, septation, conidiophore morphology, and the shape, size, color, and arrangement of conidia (transverse and longitudinal septa)-were thoroughly examined and documented at 400× magnification.

Results and Discussion.

Within 3 to 5 days post-inoculation, fungal growth consistently emerged from the margins of the plated tissue segments on PDA media. Initial colony development displayed whitish-grey aerial mycelium, which progressively matured into dark olive-green, brown, to nearly black colonies with a characteristic velvety or woolly texture and a dark reverse pigmentation, matching typical descriptions of *Alternaria* species.

Microscopic analysis of the slide preparations revealed critical diagnostic features of the genus:

1. Mycelium: Well-developed, septate, branched, and heavily pigmented (dematiaceous hyphae ranging from light brown to deep olivaceous).

2. Conidiophores: Short, often solitary or aggregated, septate, arising directly from the mycelium.

3. Conidia (Asexual Spores): Large, dark-colored (muriform) conidia possessing both transverse and longitudinal septa. They exhibited typical obclavate, pear-like, or

ovoid shapes with distinct tapered apical beaks, frequently found arranged in short or branched chains.

These morphological markers closely align with standard taxonomic keys for *Alternaria* identification. The successful recovery of viable pure cultures confirms that the applied surface-sterilization and isolation pipeline effectively eliminates epiphytic contaminants while preserving the target pathogen.

Conclusion.

In conclusion, an efficient protocol for isolating, purifying, and morphologically identifying the phytopathogenic fungus *Alternaria* from infected host tissues was successfully demonstrated. The integration of surface sterilization, selective transfer, and detailed light microscopy allowed reliable confirmation of the isolate's identity down to the genus level based on characteristic dematiaceous septate hyphae and muriform conidial structures. The resulting pure cultures represent a vital biological asset (collection object) for future physiological testing, screening of biological antagonists, and molecular pathology studies aimed at mitigating alternarioz diseases in agriculture.

References

1. Simmons, E. G. (2007). *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Centre, Utrecht, Netherlands.
2. Rotem, J. (1994). *The Genus Alternaria: Biology, Epidemiology, and Pathogenicity*. American Phytopathological Society (APS Press), St. Paul, Minnesota.
3. Thomma, B. P. H. J. (2003). *Alternaria* spp.: from general pathogen to major industrial hazard. *Molecular Plant Pathology*, 4(4), 225-236.
4. Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., & Crous, P. W. (2013). *Alternaria* redefined. *Studies in Mycology*, 75, 171–212.