Microbial Deconstruction Of Cellulosic Cotton Fabric: Isolation, Identification And Morphological Confirmation Of Fungal Biodegradation

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Abstract

The present investigation focuses on the isolation, identification, and confirmation of fungal degradation of cotton fabric under humid storage conditions. Degraded cotton samples were collected from textile units in Jaipur and Surat and screened for fungal contamination using Sabouraud Dextrose Broth. Five dominant fungal strains were isolated—Aspergillus flavus, Lichtheimia ramosa, Penicillium oxalicum, Aspergillus nidulans, and Aspergillus niger. Cellulolytic activity was evaluated through qualitative and quantitative assays, including CMC agar plate, DNSA, and filter paper assays. The relative cellulase activity, measured by optical density at 540 nm, indicated significant reducing sugar release, with A. flavus exhibiting the highest activity. SEM analysis confirmed structural damage to cotton fibers post fungal exposure, correlating with enzyme-mediated cellulose hydrolysis. This study demonstrates the biodegradative potential of fungal species in humid textile environments, contributing to an improved understanding of microbial deterioration of cotton-based materials.

1. Introduction

Cotton is one of the most widely used natural textile fibers due to its comfort, biodegradability, and high cellulose content.(1) However, the cellulose-rich nature of cotton makes it highly vulnerable to microbial attack, particularly by fungi under warm and humid storage conditions. Fungal contamination of cotton fabrics results in mildew formation, unpleasant odor, discoloration, and significant deterioration of mechanical properties.(2,3) Such biodegradation not only causes economic losses in the textile industry but also reduces the durability and usability of cotton-based materials.(4)

Fungi are known to secrete extracellular cellulolytic enzymes, including endoglucanases, exoglucanases, and β-glucosidases, which collectively hydrolyze cellulose into soluble sugars. (5) The extent of cotton degradation depends on fungal species, environmental conditions, and duration of exposure.(6) Several genera such as *Aspergillus*, *Penicillium*, and *Lichtheimia* are frequently associated with textile biodeterioration. Understanding their enzymatic activity and fiber degradation mechanisms is essential for developing preventive strategies.(7,8)

Therefore, the present study focuses on the isolation and identification of dominant fungal strains from mildew-affected cotton fabrics and evaluates their cellulolytic potential using enzymatic assays and morphological analysis.(9) This work contributes to a better understanding of microbial deconstruction of cotton fibers in humid textile environments.(10,11)

2. Materials and Methods

a. Isolation and identification:

Degraded cotton fabric samples were collected from Jai Texart, Jaipur, and selected local textile units in Surat, Gujarat. The samples were aseptically transferred to sterile containers and processed immediately for microbial analysis. Fungal isolation was carried out by incubating fabric pieces in Sabouraud Dextrose Broth at 28 ± 2 °C for 5–7 days. After visible fungal growth, aliquots were streaked onto Sabouraud Dextrose Agar plates to obtain isolated colonies. Distinct fungal colonies were repeatedly sub-cultured to achieve pure cultures.(12)

Preliminary identification of fungal isolates was performed based on macroscopic colony morphology, pigmentation, and growth patterns. Microscopic examination was conducted using lactophenol cotton blue staining to observe spore and hyphal structures. For molecular identification, genomic DNA was extracted from pure cultures and amplified using ITS region primers. The obtained sequences were compared with existing sequences in the NCBI database for species confirmation. Accession numbers were assigned to the identified fungal isolates for reference and validation.

b. Weight loss measurement:

Cotton fabric samples were cut into equal-sized pieces, dried, and their initial weights were recorded. The samples were inoculated with individual fungal isolates and incubated under controlled humid conditions for a defined period. After incubation, the fabrics were gently cleaned to remove surface mycelia and dried to constant weight. Final weights were recorded, and percentage weight loss was calculated to assess the extent of fungal degradation. Uninoculated fabric served as the control for comparison.

c. Tensile strength measurement:

Cotton fabric samples were inoculated separately with different fungal isolates and incubated under humid conditions for a fixed duration. After incubation, the samples were carefully cleaned and dried to constant weight. Tensile strength was measured using a standard tensile testing method by applying load until fabric breakage. The tensile strength values were

calculated in MPa and compared with untreated control samples. Reduction in tensile strength was used as an indicator of fungal-induced fiber degradation.

d. SEM images of cotton fabric:

Untreated and fungal-treated cotton fabric samples were first cut into small, uniform pieces and gently washed with sterile distilled water to remove loosely attached debris and mycelial residues. The samples were then fixed using 2.5% glutaraldehyde in phosphate buffer to preserve surface morphology, followed by thorough rinsing with the same buffer. Dehydration was carried out through a graded ethanol series (30–100%) to remove moisture without damaging fiber structure.

The dehydrated samples were air-dried and mounted on aluminium stubs using carbon adhesive tape. To enhance conductivity, the samples were sputter-coated with a thin layer of gold. Surface morphology of untreated and fungal-treated cotton fabrics was examined using a scanning electron microscope operated at 15 kV and 500× magnification. Structural changes such as fiber erosion, cracks, fibrillation, and hyphal penetration were recorded and compared with the untreated control.

3. Results:

Isolation and identification:

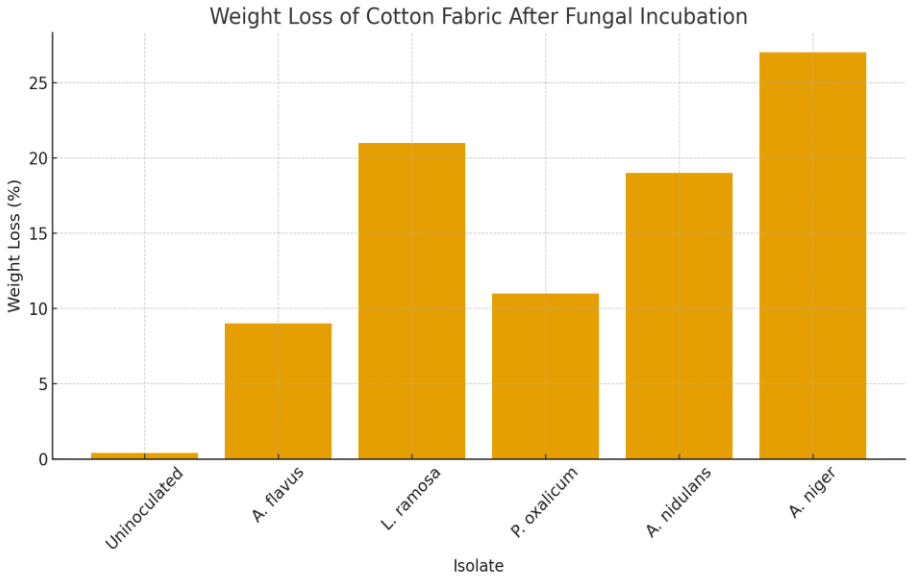
The table summarizes the molecular identification, growth characteristics, and microscopic morphology of the fungal isolates recovered from degraded cotton fabric. NCBI accession numbers confirm species-level identification, while cultural and microscopic features support their taxonomic classification.

Table 1. Morphological and Molecular Identification of Fungal Isolates

Strain	Species	NCBI Accession	Growth Characteristics	Microscopic Morphology
K1	Aspergillus flavus	Pq285820	White, velvety, yellow-green conidia	Globose conidia 250–450 µm
K2	Lichtheimia ramosa	Pq285863	Woolly, gray-brown colonies	Aerial mycelia, sporangiospores
K3	Penicillium oxalicum	Pq285824	Velvety, dark green colonies	Septate hyphae, branched conidiophores
K4	Aspergillus nidulans	Pq286033	White to dark green, wool-like	Short conidiophores, rough conidia
K5	Aspergillus niger	Pq286034	Filamentous, black conidia	Smooth conidiophores, biseriate heads

The results confirm the successful isolation and identification of five dominant fungal species associated with cotton fabric degradation. Molecular identification using NCBI accession numbers corroborated the morphological and microscopic observations. These fungi are known cellulolytic organisms, indicating their significant role in cotton biodeterioration.

Weight loss measurement:

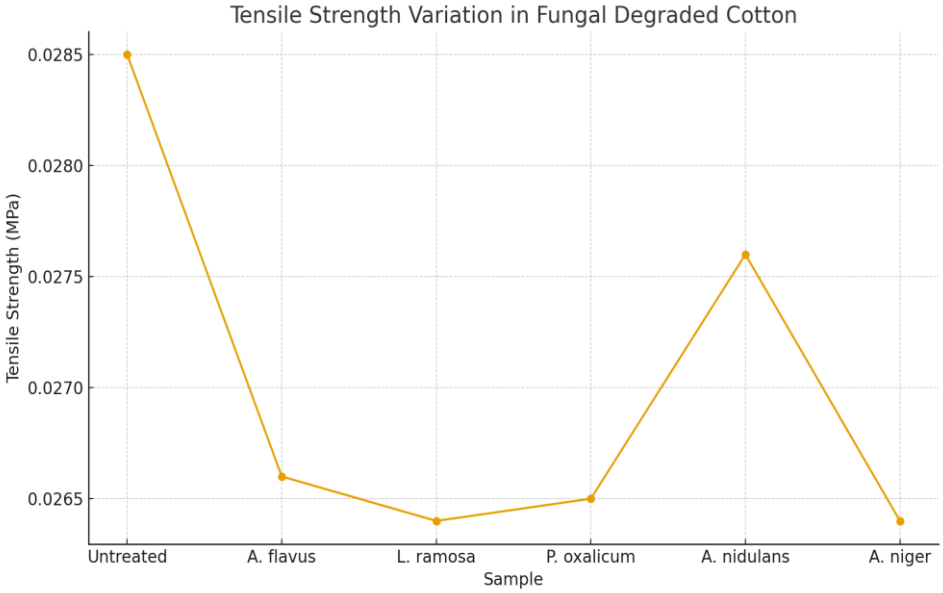


Graph 1. Weight loss percentage of cotton fabric after 20 days of fungal incubation.

The graph illustrates the percentage weight loss of cotton fabric after incubation with different fungal isolates. The uninoculated control shows negligible weight loss, confirming fabric stability in the absence of fungi. Among the tested fungi, *Aspergillus niger* caused the highest weight loss, indicating strong cellulolytic and degradative activity. *Lichtheimia ramosa* and *Aspergillus nidulans* also produced substantial fabric degradation. *Penicillium oxalicum* and *Aspergillus flavus* showed comparatively lower but noticeable weight loss. Overall, the results demonstrate varying degrees of fungal-mediated cotton fabric degradation.

Tensile Strength measurement:

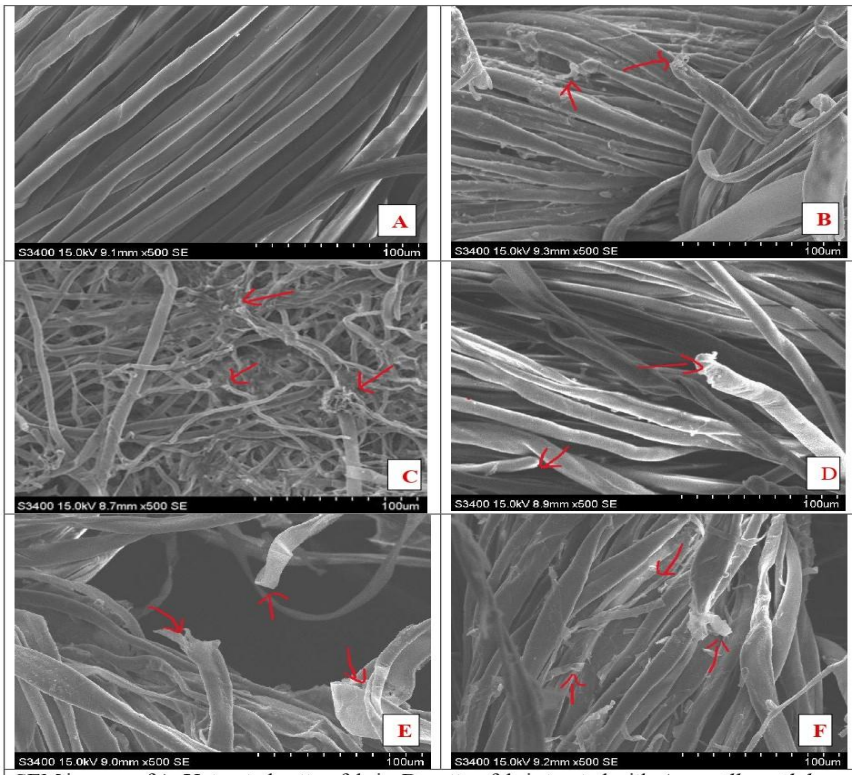
The tensile strength of cotton fabric after fungal incubation was evaluated to assess the extent of mechanical deterioration caused by different fungal isolates. The untreated fabric served as a control for comparison with fungal-degraded samples.



Graph 2. Variation in tensile strength of cotton fabric after fungal degradation.

The graph shows variations in tensile strength of cotton fabric following fungal degradation. The untreated sample exhibits the highest tensile strength, indicating intact fiber integrity. All fungal-treated samples show a reduction in tensile strength, confirming deterioration of the cotton fibers due to fungal activity. *Lichtheimia ramosa* and *Aspergillus niger* cause the greatest strength reduction, suggesting aggressive cellulose degradation. *Aspergillus nidulans* shows comparatively higher tensile strength among treated samples, indicating less damage. Overall, fungal incubation significantly compromises the mechanical strength of cotton fabric.

SEM Analysis:



SEM images of A: Untreated cotton fabric, B: cotton fabric treated with *Aspergillus nidulans*, C: cotton fabric treated with *Aspergillus niger*, D: cotton fabric treated with *Penicillium oxalicum*, E: cotton fabric treated with Lichtheimia ramosa, F: treated with *Aspergilluss flavus*.

SEM micrographs revealed clear structural alterations in fungal-treated cotton fibers. Uninoculated samples showed smooth, intact fibers (~200 µm), whereas inoculated samples exhibited ruptured and fibrillated structures, indicative of enzymatic cellulose degradation. Aspergillus niger caused the most severe damage among all isolates.

4. Discussion

The study demonstrates that cellulolytic fungi, particularly Aspergillus species, are capable of degrading cotton fibers by secreting hydrolytic enzymes. DNSA-based cellulase quantification confirmed reducing sugar release, supporting enzymatic cellulose hydrolysis. Environmental humidity and prolonged storage create favorable conditions for fungal colonization, leading to textile deterioration.

5. Conclusion

The isolation and identification of cellulolytic fungi from mildew-affected cotton confirm their significant role in textile biodegradation. The combination of biochemical assays, tensile strength reduction, and SEM analysis provided comprehensive evidence of enzymatic degradation of cellulose in cotton fibers.

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