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Identification of Pathogenic Fungi from Wet-Blue and its Inhibition using

Citronella Oil Nanoencapsulates as Ecofriendly Antifungal Agent

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Abstract

Microbial infestation of wet leathers during its storage and transport is major challenge in the tanning industry leading to structural and aesthetic degradation, compromised leather quality, reduced commercial value and reliance on synthetic thiazolebased fungicides. To address these issues, the present work focuses on identifying two fungal pathogens responsible for imparting color and biodetoriating wetblue and the development of an essential oil based antifungal agent to impart antifungal functionality to the leathers. The contaminated wetblue samples were collected from Calcatta Leather Complex, Bantala and the dominant fungal strains responsible for imparting color were isolated under aseptic conditions. Identification was confirmed using morphology and DNA sequencing, revealing the fungi to be Candida palmiloephila P-18 and Candida palmiloephila CBS 7418. For development of the antifungal agent, Citronella oil was encapsulated using silica to form nano/micro-capsules ranging from 100-1000 nm through emulsion polymerization techniques. The product was then characterized for hydrodynamic diameter (DLS), surface charge (Zeta potential), functional groups (FT-IR), thermal stability and loading efficiency (TGA and DSC) and surface morphology (SEM and TEM). The antifungal activity

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of the encapsulated citronella oil was studied using agar-well diffusion method. The product was applied on the leather and the evaluated for the antifungal activity showed highly pronounced results, depicting complete resistance of growth. Thus, this bio based antifungal product is a better solution to address the problems associated with infestation of unknown pathogenic fungi rather than using conventional fungicides.

Keywords: Wet-blue, Fungi, DNA sequencing, Essential oil, Candida palmiloephila, Antifungal agent.

1. Introduction

Wet-blue leather, an important intermediate material in the leather manufacturing chain, is widely traded and stored prior to further processing. However, due to its high moisture content and proteinaceous collagen matrix, wet-blue leather is particularly susceptible to fungal contamination during storage and transportation [1]. Fungal growth is frequently observed under warm and humid conditions, leading to quality deterioration and reduced commercial value of leather products [2]. Pathogenic fungi colonizing wet-blue leather are responsible for defects such as discoloration, surface weakening, unpleasant odor formation, and loss of mechanical integrity [3]. In addition to material damage, fungal contamination poses occupational health concerns

for workers handling infected leather [4]. Conventional preservation practices rely on synthetic fungicides, which, despite their effectiveness, are increasingly restricted due to environmental persistence, toxicity, and the risk of microbial resistance development [5]. This has created a pressing need for safer and sustainable antifungal alternatives for leather preservation. Essential oils obtained from plant sources have attracted significant attention as natural antifungal agents owing to their broad-spectrum activity, biodegradability, and lower environmental impact [6]. Their antifungal efficacy is primarily attributed to bioactive constituents such as

terpenes and phenolic compounds, which disrupt fungal cell membranes and inhibit metabolic activity [7]. Nevertheless, issues related to volatility and stability limit their direct application in industrial systems. The present work systematically identifies pathogenic fungi specifically associated with imparting color to wet-blue leather, followed by the development of a formulated essential oil-based antifungal system tailored for leather preservation. This integrated approach bridges microbiological characterization with sustainable formulation development, offering a practical and eco-friendly alternative to conventional synthetic fungicides for wet-blue leather.

2. Methodology

2.1 Isolation and identification of fungi from wet-blue leather

Fungal-contaminated wet blue leather samples with distinct pigmentation were collected from tanneries at the Calcutta Leather Complex, Bantala. Surface fungal spores were microscopically observed and aseptically cultured on PDA supplemented with chloramphenicol, followed by incubation at 30 °C. Successive isolation was achieved through serial dilution and repeated subculturing. Pure fungal colonies were maintained at 4 °C with periodic subculturing. Morphological identification was performed using lactophenol cotton blue–stained slides under a compound microscope, based on hyphal and colonial characteristics [8]. Isolates were further differentiated using CHROMagar, with observations recorded up to 21 days of incubation.

2.2 DNA isolation and DNA sequencing of fungal DNA

Fungal biomass from PDA-grown colonies was harvested and subjected to genomic DNA extraction using a CTABbased method. PCR products were resolved on 2% agarose gel and visualized under UV illumination. Purified amplicons were Sanger sequenced, and species-level identification was achieved by sequence alignment using BLASTN against the NCBI database.

2.3 Formulation of Citronella oil encapsulates (Product) and Antifungal activity of Citronella oil and formulated product

Citronella oil was emulsified with CTAB in deionised water at 5000 rpm and encapsulated in silica using TEOS further surface modified by ATPES. Antifungal efficacy of the citronella oil and formulated encapsulated product was carried out against the identified fungal strains WBY and WBP using potato dextrose agar plates under static and humid conditions at 27°C, and observations were made on the 7th, 14th and 21st day. Minimum inhibitory concentration was calculated accordingly.

2.4 Application in Leather and Antifungal activity of the infused leather

To evaluate the antifungal sensitivity of the citronella oil encapsulates in leather, wet salted goat skins were taken as raw material. The operations done on the control and the infused (experimental) leathers. Antifungal efficacy of the citronella oil encapsulate infused leather was carried out against the identified fungal strains WBY and WBP.

3. Results and discussion

3.1 Isolation and identification of fungi from wet-blue leather

Pigmented fungal growth on wet-blue leather samples, colony morphology on PDA plates and optical microscopic observation, the isolated organisms was identified as yeasts and were termed as WBY and WBP respectively. Further confirmation of the fungal genus was carried out using

CHROMagar Candida plates. The distinct colony coloration observed on the CHROMagar medium confirmed that the isolates belonged to the genus Candida. However, the chromogenic response did not correspond to any commonly reported Candida species previously associated with leather deterioration. Therefore, to achieve precise species-level identification, genomic sequencing of the isolate was undertaken. The fungal isolate obtained from the pinkish wet-blue sample (WBP) showed highest sequence similarity with Candida palmioleophila strain CBS 7418, while the isolate from the yellowish wet-blue sample (WBY) matched Candida palmioleophila strain P-18. Importantly, the identification of C. palmioleophila, a less frequently reported Candida species on leather substrates, underscores the limitations of phenotypic methods alone and highlights the necessity of ITS sequencing for accurate identification of atypical yeast species associated with wet-blue leather deterioration.

3.2 Antifungal Activity of Citronella Oil and Infused Leather

The antifungal efficacy of citronella oil was evaluated against the identified yeast strains WBP and WBY using an agar well diffusion assay. The minimum inhibitory concentration (MIC) for both WBP (Candida palmioleophila strain CBS 7418) and WBY (Candida palmioleophila strain P-18) was determined to be 1.56 µL, reflecting comparable susceptibility of the two isolates to citronella oil. The antifungal performance of citronella

oil–encapsulated, infused leather was evaluated against the fungal strains WBP and WBY using a contact-based agar assay. Control leather samples exhibited visible fungal growth from the initial observation period, with progressive colonization observed on both the leather surface and adjacent agar. In contrast, the citronella oil–encapsulate infused leather showed complete inhibition of fungal growth throughout the incubation period, with no visible colonies detected at any observation point. The sustained antifungal effect over 21 days indicates prolonged activity of the encapsulated citronella oil, demonstrating its effectiveness in preventing Candida palmioleophila growth on leather substrates and its potential application as a durable antifungal treatment for wet-blue leather.

Conclusion

This study highlights the persistent challenge of fungal contamination in wet-blue leather, arising from atmospheric spores introduced during processing and favored by acidic pH and oil-rich substrates [10]. A rare and less-reported yeast, Candida palmioleophila strain P-18 (WBY), along with C. palmioleophila CBS 7418 (WBP), was successfully identified, with the former exhibiting aggressive pigmentation and tolerance to conventional industrial fungicides. Citronella oil demonstrated strong antifungal activity against both isolates, with an identical MIC of 1.56 µL, indicating high efficacy at low doses. Furthermore, leather infused with citronella oil encapsulates showed complete inhibition of fungal growth over 21 days, while untreated control leather exhibited visible contamination from the initial stages. These findings establish citronella oil– based systems as effective, sustainable alternatives to synthetic fungicides for long-term protection of leather against resistant fungal species.

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