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Identification, Removal, and Preventive Protection Against Mold Contamination on Historical Photographic Negatives from the Xi'an Beilin Museum

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Abstract

The Xi'an Beilin Museum preserves a large collection of archeological photographic negatives and films dating from the 1950s to the early 1980s. These images document significant archeological discoveries, including Tang dynasty imperial tomb murals, the excavation of the terracotta warriors, and various historical grottoes and stone carvings. As unique visual records of cultural heritage, these materials provide valuable references for studying environmental deterioration processes and for guiding conservation and restoration practices. However, long-term storage under uncontrolled environmental conditions has resulted in severe degradation of the negatives, including mold contamination, emulsion layer powdering, deformation, and partial detachment. Among these deterioration phenomena, microbial growth is particularly destructive because fungal hyphae cause light scattering and image obscuration, preventing scanning and digital archiving. In this study, mold species present on the negatives were isolated and identified using morphological observation and ITS rDNA sequence analysis. Based on the characteristics of the microbial contamination, targeted removal and restoration treatments were applied to recover the original image information. Furthermore, preventive protection strategies were implemented through the development of antifungal storage materials and protective containers. The results establish an integrated conservation approach combining microbial identification, restoration treatment, risk elimination, and preventive protection, providing a scientific basis for the long-term preservation of historical photographic archives.

Keywords: photographic negatives; mold contamination; preventive conservation; antifungal materials; cultural heritage preservation

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1. Introduction

The Xi'an Beilin Museum preserves an important collection of archeological photographic records dating from the 1950s to the 1980s. The archive includes more than 60,000 photographic negatives, 380 glass plate negatives, and 29 motion picture films documenting major archeological excavations and cultural heritage sites in Shaanxi Province. These images record the original appearance of archeological sites, ancient architecture, and unearthed artifacts, including panoramic views of Tang dynasty imperial tombs, murals from the tombs of Princess Yongtai and Princes Zhanghuai and Yide, as well as the

excavation process of the terracotta warriors and bronze chariots from the Mausoleum of Qin Shi Huang [1].

As irreplaceable historical documentation, these photographic materials provide critical references for studying the deterioration processes of cultural heritage and for guiding restoration and conservation projects [2–4]. Photographic negatives are typically composed of multilayer structures, including cellulose acetate or nitrate film bases and gelatin emulsion layers containing silver halide particles [5,6]. However, when these materials were originally archived, they were stored in ordinary storage environments without temperature or humidity control. Over decades of storage, the negatives were affected by both intrinsic material instability and environmental factors, resulting in severe deterioration phenomena such as mold contamination, silver mirroring, and acetic acid syndrome [7–9].

In particular, mold growth has become one of the most serious threats to the preservation of these negatives. The original packaging materials, mainly sulfate paper and kraft paper envelopes, have undergone significant aging and acidification and no longer provide effective protection against microbial attack. As a result, fungal colonization has occurred on both the base layer and the gelatin emulsion layer of the negatives [10–13], leading to image corrosion, information loss, and difficulties in digitization.

In recent years, the deterioration of photographic materials has attracted increasing attention in the field of cultural heritage conservation. Photographic negatives, particularly those produced before the 1980s, commonly consist of cellulose acetate or nitrate film bases combined with gelatin emulsion layers containing silver halide particles. These complex multilayer structures are highly sensitive to environmental conditions such as humidity, temperature, and biological contamination. Once exposed to high humidity environments, microorganisms such as fungi and bacteria can easily colonize the gelatin binder and initiate biodeterioration processes. The most frequently reported fungal genera on photographic and archival materials include Aspergillus, Penicillium, Cladosporium, and Trichoderma [14,15].

Fungal contamination has been widely recognized as one of the most destructive biological factors affecting photographic archives. Fungal hyphae can penetrate the gelatin matrix, leading to enzymatic degradation of proteins and the production of organic acids that further accelerate chemical deterioration. In addition, the presence of fungal colonies causes optical scattering on the surface of photographic materials, resulting in image blurring and loss of visual information. Previous studies have reported that common fungal genera responsible for photographic deterioration include *Aspergillus*, *Penicillium*, *Cladosporium*, and *Trichoderma*, which can rapidly proliferate under conditions of high relative humidity (>65%) and moderate temperature. Various preventive conservation strategies have been proposed, including environmental control, antimicrobial coatings, and the application of functional nanomaterials [16–19].

To mitigate these risks, conservation strategies for photographic archives generally involve two complementary approaches: restoration treatments and preventive conservation. Restoration methods aim to remove existing microbial contamination and stabilize damaged surfaces, while preventive conservation focuses on improving storage environments and developing antifungal materials for long-term protection. Recent advances in materials science have introduced new possibilities for preventive conservation, including the use of inorganic nanoparticles, antimicrobial coatings, and functional storage materials that inhibit microbial growth while maintaining chemical stability.

However, despite these advances, systematic studies focusing on the identification, removal, and preventive protection against mold contamination on historical photographic negatives remain limited, particularly for large archeological photographic collections in China. Therefore, the present study investigates mold contamination on photographic

negatives preserved in the Xi'an Beilin Museum and proposes an integrated conservation strategy combining microbial identification, restoration treatment, and preventive storage protection [5,6].

To address these problems, this study focuses on: the identification of mold species colonizing the photographic negatives; the development of restoration treatments for mold removal; and the design of preventive protection strategies.

2. Materials and Methods

2.1. Mold Identification

2.1.1. Sample Collection

The experimental samples were collected from mold-contaminated photographic negatives preserved in the Xi'an Beilin Museum (Figure 1). Due to the lack of environmental control in the early storage conditions, temperature and humidity fluctuated with the outdoor environment, resulting in extensive microbial growth. Mold colonies were widely distributed on the surface of the negatives, with particularly severe contamination observed along the edges of the film.

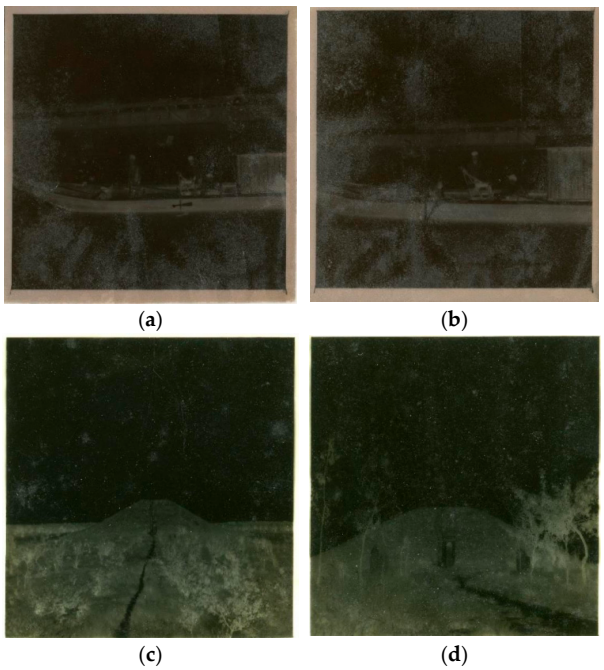


Figure 1. Mold in the emulsion layer of photographic plates from the Xi'an Beilin Museum. (**a**–**d**) Mold in the emulsion layer of photographic plates.

2.1.2. Reagent

Potato glucose agar medium (PDA Beijing Aoboxing Biotechnology Co., Ltd., Beijing, China); lactic acid phenol cotton blue dyeing agent (Beijing Solaibao Technology Co., Ltd., Beijing, China); inoculation ring (Beijing Unionic Biotechnology Co., Ltd., Beijing, China); nanometer zinc oxide (Shanghai Maclin Biochemical Technology Co., Ltd., Shanghai, China); the primer (Table 1) was synthesized by Tsingke, Shanghai, China.

PDA Medium: A total of 46 g of PDA is added to 1000 mL of deionized water, stirred with a magnetic mixer, and heated. After boiling, the solution is quickly divided and poured into a conical bottle, and then the bottle is sealed with a sterile permeable bottle film. Finally, the conical bottle is placed into a pressure steam sterilization pot and sterilized at 121 °C for 15 min. The sterilized PDA solution is poured into a disposable Petri dish—20~30 mL is poured into each Petri dish—and the solution is cultured in a mold incubator for 24 h, after which it is completely condensed, and no mold growth can be observed.

Table 1. General primers for fungal species identification.

Category	Title	Sequence 5′→3′	Amplified Sequence	PCR Length/bp
Fungi	ITS1 ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	ITS	750 bp

2.1.3. Main Instrument

SW-CJ ultra-clean UV workbench (Tianjin Xinbo Instrument Co., Ltd., Tianjin, China); DX-280B pressure steam mold cooker (Shanghai Shen 'an Medical Equipment Factory, Shanghai, China); MJP mold incubator (Beijing Zhongxing Weiye Instrument Co., Ltd., Beijing, China); model ME204E electronic balance (METTLER Toledo Instrument Co., Ltd., Switzerland); pipette gun (100 μL, 1000 μL, GILSON KHB, Paris, France); and XWY-VIII fiber analyzer (Beijing Lunhua Technology Co., Ltd., Beijing, China).

2.1.4. Isolation and Purification of Fungal Strains

Mold samples were collected from contaminated areas of the negatives using sterile swabs moistened with physiological saline. The collected spores were streaked onto potato dextrose agar (PDA) plates and incubated at 28 °C and 80% relative humidity for 5–7 days. Colonies with distinct morphological characteristics were selected and subcultured repeatedly until pure strains were obtained.

2.1.5. Morphological Identification of Mold

A small amount of mycelium was picked up on the surface of isolated and purified colonies with an inoculum needle and placed on conductive adhesive. The morphology of mycelium and spores was observed and photographed by scanning electron microscope.

2.1.6. Molecular Biological Identification

Primers ITS1:5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4:5′-TCCTCCGCTTATTGATATGC-3′ were used to amplify the fungal ITS sequence. The PCR reaction system and conditions are shown in Table 2. The amplified PCR products were subjected to agarose gel electrophoresis (2 μL sample + 6 μL bromophenol blue), nucleic acid was collected, and the sequence was determined by Beijing Genko Biotechnology Co., Ltd. (Beijing, China). The gene sequences of the sequenced strains were BLAST compared in the NCBI nucleic acid database for homology analysis. The MEGA 7 software was used to compare multiple sequences, and the taxonomic status of each mildew strain was determined according to the morphological characteristics of mold [20,21].

Table 2. PCR reaction systems and reaction conditions.

Reaction Systems	Quantity/μL	Reaction Condition	
1× TSE101 Mix	45	98 °C, 2 min	
ITS1 (10p)	2	98 °C, 10 s 53 °C, 10 s 72 °C, 10 s	38 Cycle
ITS4 (10p)	2		
Template (ng/μL)	2	72 °C, 5 min	
Total		4 °C Preserve	

2.2. Microscopic Observation and Evaluation on the Elimination of Mildew Light Scattering Effect and the Reinforcement of Original Appearance

For mold removal from the film base, a corrosion method was applied. For the restoration and protection of mold-infected photographic negatives, a patented removal

process was employed (China Patent No.: ZL 201110051677.4). Additionally, a mildew masking process was used to restore the emulsion layer (Patent No.: 201510329361.5). Surface morphology and roughness parameters before and after treatment were analyzed using a laser microscope (Keyenes VK-X260K, Osaka, Japan).

2.3. Four Anti-Mold Equipment Experiments

On a clean bench, 100 μL of conidial suspension of each fungal strain was evenly spread onto PDA plates using a sterilized glass rod. Inorganic antifungal materials and special container paper were placed onto the inoculated PDA medium. The plates were incubated at 28 °C and 80% RH for 3 days to observe fungal growth. The inhibitory effect was evaluated based on the formation of inhibition zones.

3. Results and Discussion

3.1. Results of Mold Isolation, Purification, and Sequencing

Four fungal strains were isolated from the contaminated negatives and labeled as strains A, B, C, and D. Colony morphology, conidial structures, and mycelial characteristics were first examined on PDA medium, followed by molecular identification using ITS sequence analysis (Figure 2).

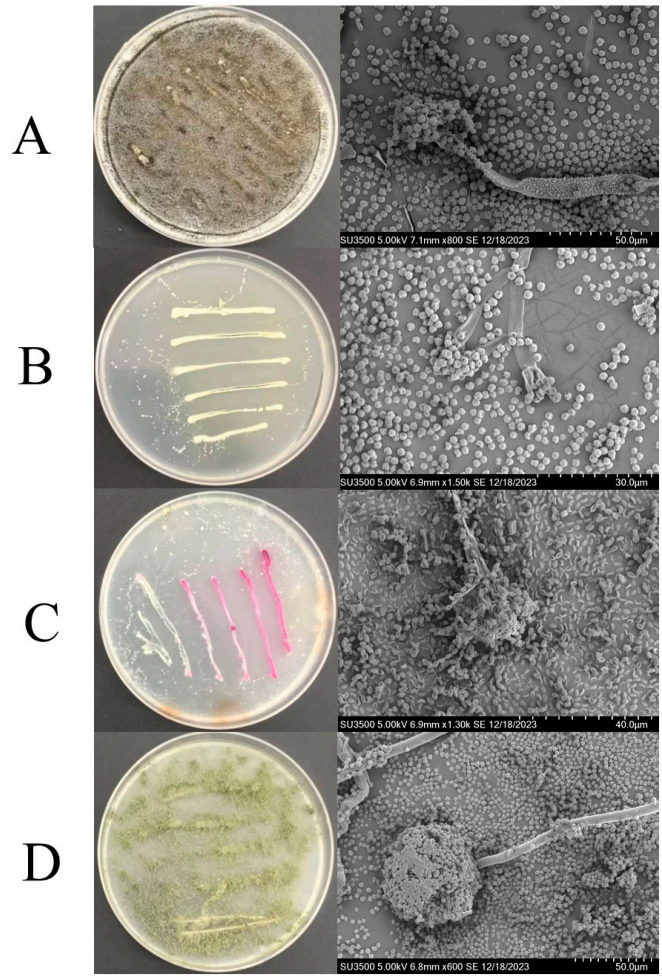


Figure 2. Morphology and microstructure of mold colonies (400×). (**A**–**D**) represents the four different forms of microorganisms that have been isolated.

BLAST comparison of the obtained ITS sequences with the NCBI database revealed high similarity with known fungal species. As summarized in Table 3, the four strains were

identified as *Aspergillus niger*, *Penicillium vinaceum*, *Trichoderma lentiforme*, and *Aspergillus flavus*. Phylogenetic trees constructed using MEGA 7 further confirmed the taxonomic classification of the isolated strains. Similar fungal species have been widely reported on photographic archives and other heritage materials [22,23].

Table 3. Blast results of the 4 mold strains.

Strain Number	The Most Homologous Strain	Entry Number	Homology
A	*Aspergillus niger*	MT628904.1	100.00%
B	*Penicillium vinaceum*	MT482619.1	99.82%
C	*Trichoderma lentiforme*	MN262489.3	100.00%
D	*Aspergillus flavus*	MT529928.1	100.00%

According to the sequencing results, the obtained gene sequence was compared with the gene sequences included in the NCBI nucleic acid sequence database for homology. The sequence with the highest score in the database was selected as the reference object, and the sequencing comparison results were shown in Table 3. According to the phylogenetic tree analysis results (Figures 3–6), combined with the morphological characteristics and homology comparison results of different strains, the phylogenetic relationships of the four strains were determined.

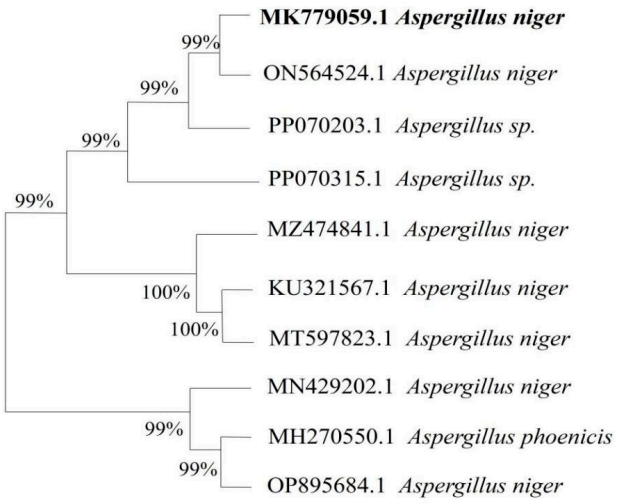


Figure 3. Phylogenetic tree of the ITS sequence of strain A.

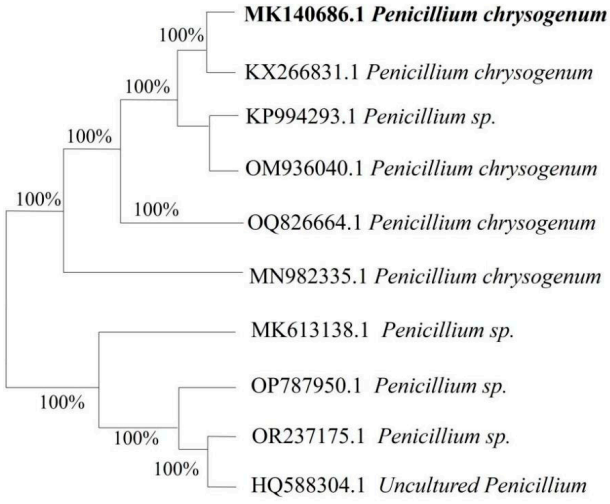


Figure 4. Phylogenetic tree of the ITS sequence of strain B.

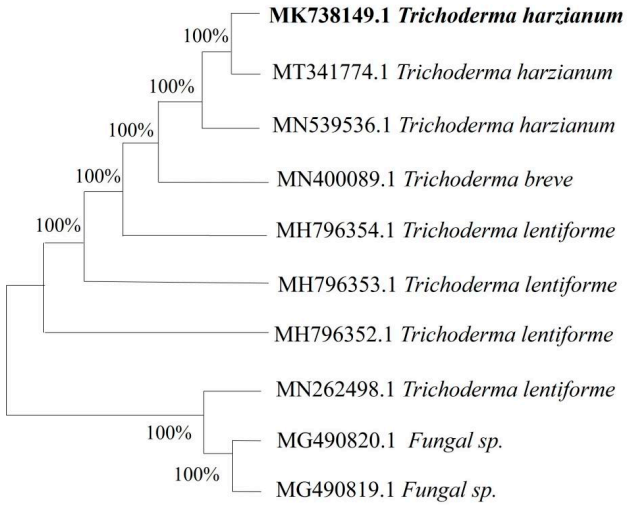


Figure 5. Phylogenetic tree of the ITS sequence of strain C.

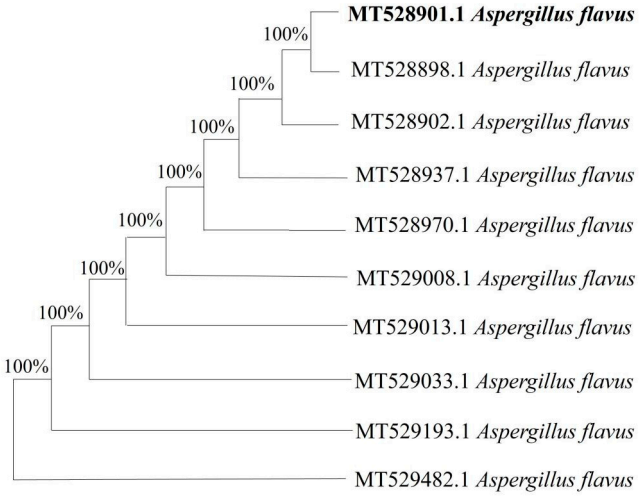


Figure 6. Phylogenetic tree of the ITS sequence of strain D.

Strain A: The growth rate is fast. After 3 days of cultivation at 28 °C, the colony almost covers the entire medium. The colony is flat and olive black on PDA medium. The colony surface is smooth and flat. The colony is initially white, often with bright yellow areas and thick velvet, and then it turns black. The back is colorless, or the central part is slightly brown. The microscopic results showed that the conidial head was spherical or radial, shaped like a chrysanthemum, and brown-black in color. The conidial stem was colorless, or the upper part was light yellow and smooth, suggesting *Aspergillus Niger*. The sequencing results showed that strain A had 100% homology with *Aspergillus niger*. Based on colony growth, conidial morphology, and sequencing results, strain A was identified as be *Aspergillus Niger* [24].

Strain B: The growth rate was fast. After 3 days of cultivation at 28 °C, the colony occupied half of the medium. On PDA medium, the colony shape was round, the surface was smooth and cyan, the colony texture was uniform and opaque, the central part was dark in color, there were spots in the middle, and the edge was irregular and covered with white mycelium. Microscopic observations showed that the top of the mycelium conidium did not form an enlarged apical sac and that the conidium branched several times, producing several rounds of symmetrical or asymmetrical small peduncle, which looked like a broom and which is called a broom body. The sequencing results showed that

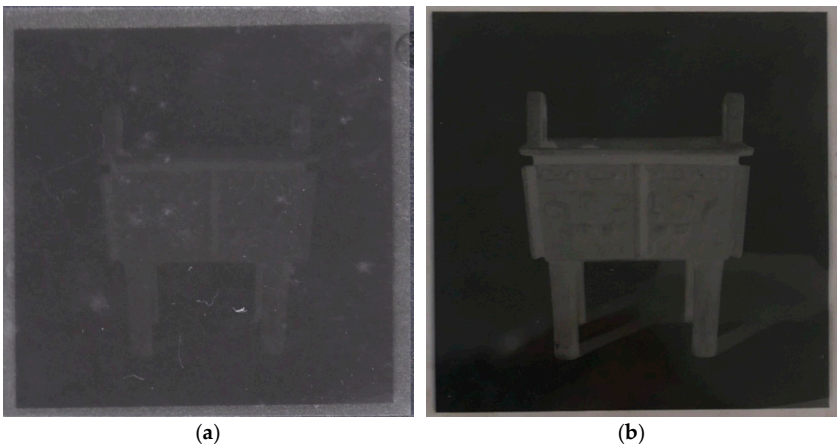
strain B had 99.82% homology with *Penicillium vinaceum*. Based on colony growth, conidial morphology, and sequencing results, strain B was identified as *penicillium* [25].

Strain C: The growth rate was fast. After 3 days of culturing at 28 °C, the colony has spread over the whole medium. In the beginning, on PDA medium, the colony was white, dense, and round, expanding to four directions. Then, green spores were produced from the center of the colony, the center became green, and there was a growth belt of white mycelia around the colony. Finally, the whole colony became green. The microscopical results showed that the conidial peduncle was vertically symmetrically divided, and the conidial conidia were solitary or clustered, round, and green. The sequencing results showed that strain C had 100% homology with Trichoderma lentiforme. Based on colony growth, conidial morphology, and sequencing results, strain C was identified as trichoderma [26].

Strain D: The growth rate was fast. After 3 days of cultivation at 28 °C, the colony covered almost the whole medium; colony growth was faster on PDA medium. The structure was loose, with a white outer layer and light green inner layer. The whole colony appeared yellowish-green. The back was colorless or slightly brown and villous. The thallus was composed of many complex branching mycelia. The microscopic results showed that the conidial head was radiate, hemispherical, or subspherical. The surface was rough, and the pedicel was colorless and rough. The apical sac was spherical, and the conidia were yellow, spherical, or nearly elliptical. The sequencing results showed that strain D had 100% homology with Aspergillus flavus. Based on colony growth, conidial morphology, and sequencing results, strain D was identified as *Aspergillus flavus*.

3.2. Scientific Evaluation of Restoration and Protection Technology of Moldy Photographic Negatives

The mildew generated by the emulsion layer of the negative and the glass negative is rooted in the gelatin, and the light-scattering effect generated by the mycelium covers the original appearance of the corroded image, which is blurred and cannot be scanned and digitized. In this paper, the "innovative technology for stabilizing materials to eliminate light scattering at micro-nano scale rough interface" created by the Key Laboratory of Photographic Image Archives Protection of the State Archives Administration of Shaanxi Normal University was adopted. The development of a hardening agent composed of non-volatile liquid stabilizers (GW505, DPOP, and 372, FEVE) was applied to eliminate light scattering generated by mold mycelia, displaying and reinforcing the effect of the original appearance (Figure 7). Surface coating technologies have been increasingly applied in the conservation of cultural heritage materials to improve stability and reduce environmental deterioration [16,21,26].



(**a**) (**b**)

Figure 7. Restoration effect of mold removal on black-and-white photographic negatives. (**a**) before treatment; (**b**) after treatment.

The experimental data were corrected and analyzed using the file analysis software VK-H1XMC. The results showed that the surface roughness of the film was significantly reduced after mold removal. As shown in Figure 8A, the surface of the mold-infected negative was densely covered with fungal hyphae. Figure 8B presents the corresponding 3D morphology and roughness parameters, revealing that the surface fluctuated between −8 and 5 μm. After the treatment (Figure 8C), the uneven interface was replaced by a smooth plane. The 3D morphology in Figure 8C shows surface fluctuations reduced to between −0.1 and 0.1 μm. The arithmetic average height (Sa), maximum height (Sz), and other roughness parameters decreased significantly, indicating effective removal of mold hyphae and spores.

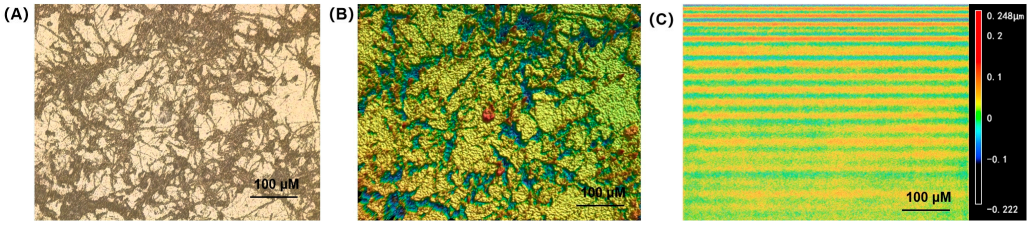


Figure 8. Surface morphology of photographic negatives before and after mold removal treatment. (**A**) 2D morphology before treatment; (**B**) 3D morphology and roughness parameters before treatment; (**C**) 3D morphology and roughness parameters after treatment.

3.3. Antifungal Efficacy of Protective Materials

In order to ensure that the repaired negatives would no longer grow mold, the bacteriostatic experiment was carried out by using environmentally friendly film-durable storage and spacer paper developed by the Key Laboratory of Photographic Image Archives Protection of the State Archives Administration of Shaanxi Normal University. The storage box and spacer paper were permeated with non-mechanical anti-mold materials such as nano-magnesium hydroxide, nano-zinc borate, sodium octaborate, etc. The dense bacteriostatic barrier was built as a whole, forming the herd immunity effect and preventing mold breeding and spreading.

In this paper, the antibacterial effect of inorganic materials and paper on four kinds of molds contaminating negative films was tested using the antibacterial circle method (Figure 9). The antibacterial circle method revealed that both inorganic materials and the treated paper exhibited strong inhibitory effects against the four isolated fungal strains. The inhibition was most pronounced against Aspergillus flavus and Trichoderma lentiforme, while Aspergillus niger and Penicillium vinaceum were also effectively suppressed. Notably, mold growth was observed on the surface of ordinary cardboard, whereas no direct contact occurred between mold and antifungal cardboard, with a clear inhibition zone visible. Inorganic antimicrobial materials such as zinc oxide and magnesium-based compounds have shown excellent antifungal performance in previous studies [19,20].

At the same time, we equipped this batch of films with disaster prevention and durable storage boxes (Figure 10), interlining paper, and strong buffer materials such as nano-magnesium hydroxide, nano-zinc borate, sodium octaborate, etc., to counteract acetic acid gas permeating the sheet and paper. Activated carbon was placed in the box under the paper lining to adsorb acid gas. The overall application of constructing a dense acid, fire, insect, and mildew barrier and network system forms a herd immunity effect, enabling the stable preservation of precious acetate films in the ordinary environments worldwide and providing a radical cure for "acetic acid syndrome" (Figure 11).

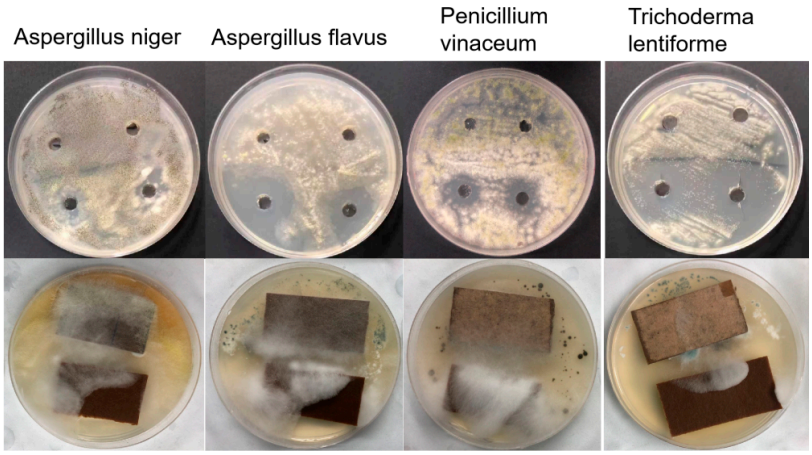


Figure 9. Antifungal activity of protective materials against mold strains isolated from photographic negatives. The images show inhibition zones around the tested materials for *Aspergillus niger*, *Penicillium vinaceum*, *Trichoderma lentiforme*, and *Aspergillus flavus*.



Figure 10. Multifunctional protective storage box for glass plate negatives.



Figure 11. Multifunctional protective storage box for film negatives.

3.4. Implications for the Conservation of Photographic Archives

The results of this study highlight the importance of integrating restoration techniques with preventive conservation strategies in the preservation of photographic archives. Mold contamination not only damages the visual information contained in photographic negatives but also accelerates chemical and structural degradation of the gelatin emulsion layer. Therefore, effective conservation approaches should simultaneously address both the removal of existing microbial contamination and the prevention of future fungal growth.

The antifungal storage materials developed in this study demonstrate promising inhibitory effects against several dominant mold species identified from the contaminated negatives. The incorporation of inorganic antimicrobial components such as nano-magnesium hydroxide and zinc borate provides a stable and environmentally friendly

approach for preventing fungal proliferation in storage environments. Compared with traditional chemical fumigation or biocidal treatments, these materials offer long-term protection without introducing additional chemical risks to the photographic materials.

Furthermore, the reduction in surface roughness observed after restoration treatment indicates that the developed coating technology effectively eliminated light scattering caused by fungal hyphae. This improvement in surface morphology significantly enhances the readability and digitalization potential of historical photographic negatives. The integration of restoration technologies with preventive storage systems therefore represents a sustainable conservation strategy for large-scale photographic collections. The integration of restoration technologies and functional protective materials represents an effective approach for the long-term preservation of photographic archives [16,21,26].

4. Conclusions

Mold contamination represents one of the most significant deterioration factors affecting photographic negative archives. Fungal growth not only produces visible mold colonies but also degrades the gelatin emulsion layer, leading to image loss, chemical degradation, and reduced mechanical stability. In this study, fungal contamination on photographic negatives from the Xi'an Beilin Museum was systematically investigated. Four dominant fungal species—Aspergillus niger, Penicillium vinaceum, Trichoderma lentiforme, and Aspergillus flavus—were isolated and identified through morphological observation and ITS sequence analysis. Restoration treatments were applied to remove fungal contamination and recover image clarity, while antifungal storage materials and protective containers were evaluated to prevent future microbial growth. The results demonstrate that the proposed restoration and preventive conservation strategy can effectively improve the preservation conditions of historical photographic negatives and reduce the risk of microbial deterioration. This study provides a practical conservation framework for the long-term preservation of photographic archives and other heritage materials susceptible to microbial damage.

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