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Isolation and identification of Microbes degrading Mural painting

Indu Singh and *Devesh Kumar

Department of Botany, Raja Balwant Singh College, Agra - 282002 (India)
Dr. Bhimrao Ambedkar University, Agra - 282004 (India)
Email: missindusingh1989@gmail.com
*Author for correspondence : drdeveshjadon@gmail.com

Abstract

Mural paintings are a particularly remarkable example of cultural expressions of humanity and play the most significant role in the understanding of societies and civilizations of our past. Murals are a unique form of artwork, which are intrinsically connected to ancient Indian painting tradition. The conservation and preservation of Indian cultural heritage is one of the main concerns. The target of this proposed research work is to find out the biodegradative phenomenon that degrades mural painting surfaces using recognizing the microbes involved in this procedure, and appraising their ecological features and deterioration capacity, to find out the best conservation strategy. The identification of the microorganisms on the paintings using standard techniques involved isolation, spread plate method, Gram staining test, biochemical tests, and slide cultures techniques.

Key words : Biodeterioration, mural painting, microorganism, conservation, cultural heritage.

One of the greatest traditions in the history of the ancient world is the mural painting heritage of India. Murals are one-of-a-kind works of art that are inextricably linked to the long history of Indian painting. The Latin word "murus," which means "wall," is the root of the English word "mural." Mural paintings are the term for the artwork created on walls[12].

Priceless knowledge about our past and cultural heritage can be found in mural art. Since they consistently feature scenes from everyday life and religious traditions that are extremely important to humanity[2]. The preservation and safeguarding of our cultural legacy is currently one of the world's top priorities, especially in India.

The transformation of the organic and inorganic composition of the substrate brought on by the growth and metabolic activity of bacteria is known as "biodeterioration of paintings," and it is a global issue[9]. According to Sarro *et al*.,[11], microbially driven degradation

destroys wall paintings by discoloring them and causing crusts to grow on their surfaces.

Other authors report the activity of microorganisms on the surface of monuments, which increases their alteration. The specialized literature reports biological attacks formed by microbes only in special cases, occurring after an increase in moisture support, as a result of infiltration, food, excessive touring, etc., or after the use of inappropriate materials in restoration[1,3,4,8,11].

The development of microorganisms in artworks could result in aesthetic and structural harm[10]. In general, humidity, a pH that is somewhat alkaline, and the presence of both organic and inorganic nutrient sources encourage the growth of microbes on mural paintings[13]. As a result of the mural murals' inherent porosity, airborne microorganism spores cause artworks surfaces that are susceptible to these propagules[14].

Due to their high capacity for biodeterioration, fungi are among the most harmful organisms associated with the biodeterioration of organic and inorganic materials in mural paintings and present a significant challenge to the preservation of cultural heritage[7]. Deterioration is the gradual loss of a structure's structural integrity due to external causes or material leakage[15].

Phototrophic organisms typically start the biofilm formation on a clean stone surface because they give an organic source for the growth of heterotrophic organisms[17].

Biodeterioration of mural painting is the result of interactions between microorganisms, nutrient support, and environmental factors[5].

This study examines the effects of biodeterioration on mural paintings from several locations in Uttar Pradesh. This study was conducted to better understand the morphological and physiological characteristics of biological agents, which are required to correctly identify the biological species that have colonized the surfaces or interiors of mural-making materials. Using isolation, scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy microbiology techniques, the microbes damaging the mural paintings were discovered and characterized (FTIR).

This investigation forms a portion of an undergraduate thesis.

Sample collection :

On one day, a total of ten samples were randomly taken from two different buildings. We looked for poor color, bad odor, viscosity loss, and biodegradation in these structures. Using a fresh, sterilized blade and a sterile sampling bottle with labeled samples were obtained by scraping the damaged mural-painted walls. These samples were hurriedly delivered to the lab for examination.

Sample preparation and analysis for bacteria :

In a conical flask, one gram (1g) of each sample from the various study sites was dissolved in 10 ml of sterile distilled water. The digested slurry sample and the fresh sample were serially diluted up to 10^{-6} tubes. The 10^{-6}

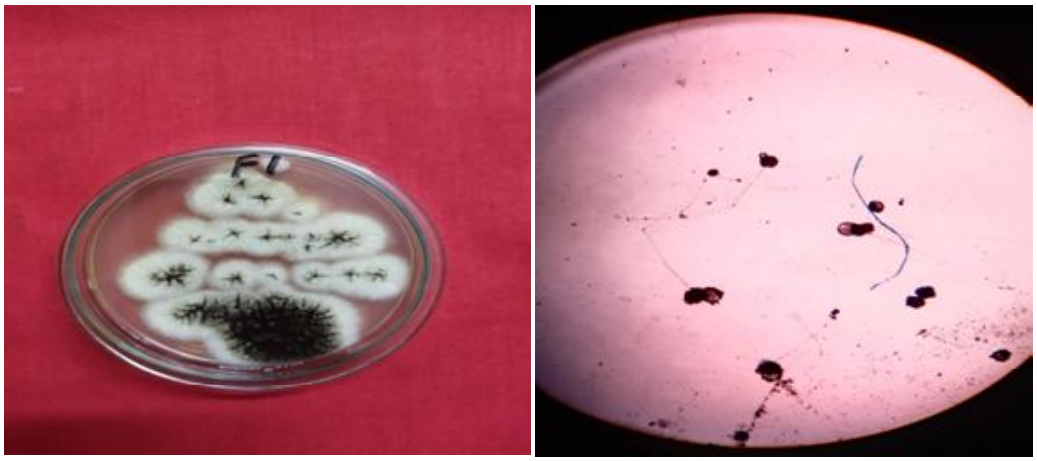

Figure 1. Colony morphology and microscopic view of *Aspergillus niger*

test tube's exact 0.1 ml was drawn out using a sterile syringe and infected utilizing the spread plate and pour plate inoculation methods onto nutrient agar plates that were already prepared. The inoculation plates were incubated for 24 hours at 37 degrees C. Colony forming units (CFU) per gram of the sample were calculated based on the number of bacterial colonies that formed on the plates. To create pure isolates, the colonies were repeatedly sub-cultured on new plates. The pure bacterial isolates were dyed with Gram stain and put through.

Isolation of fungi :

The 10^{-6} tube's aliquot (0.1 ml) was taken out using a syringe and inoculated onto already-made potato dextrose agar (PDA), which was then stored at room temperature and kept in the dark for roughly 4 to 10 days. Following that, different growth colonies were sub-cultured by spot inoculation on brand-new, sterile PDA plates to get pure culture. Another 4 to 10 days were spent growing these at room temperature. The identification process was based on morphological and colony traits, as well as on previous researchers' descriptions of the lactophenol cotton blue and slide culture procedures.

Table-1. shows the Bacterial species isolated from different sampling.

Sampling site	Bacteria isolated
A	*E. coli*
B	*Staphylococcus intermedius*
C	*Micrococcus luteus*
D	*Pseudomonas putida*
E	*Streptomyces* species
F	*Proteus vulgaris*
G	*Serratia marcescens*
H	*Aeromonas caviae*

A, B, C, D, E, F, G, and H were different sampling sites.

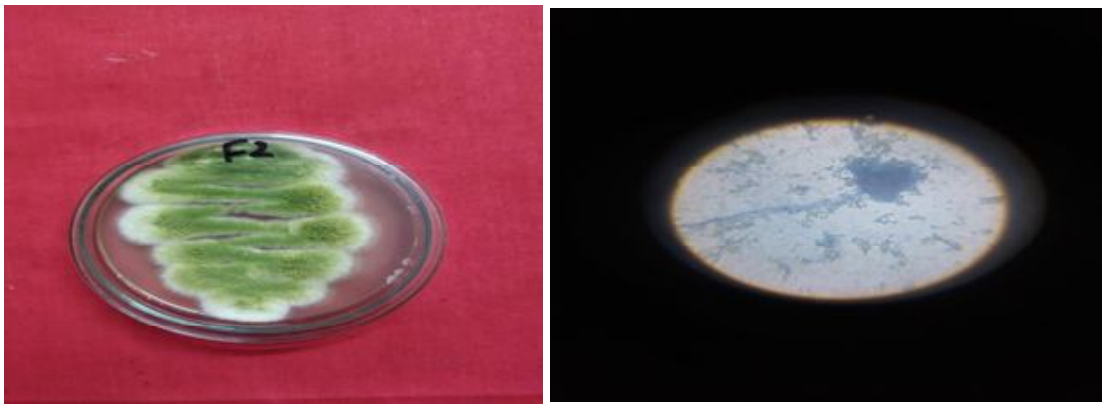

Figure 2. Colony morphology and microscopic view of *Aspergillus flavus*

Table-2. shows the Fungal species isolated from different sites.

Sampling site	Fungi isolated
F1	*Aspergillus niger*
F2	*Aspergillus flavus*
F3	*Aspergillus candidus*
F4	*Aspergillus fumigatus*
F5	*Penicillium* species
F6	*Mucor* species
F7	*Rhizopus* species
F8	*Fusarium* species

F1, F2, F3, F4, F5, F6, F7 and F8 were different sampling sites.

Table-1 shows the bacterial load of the various samples. Many bacterial species were found in various samples which were taken from different areas.

Table-2 presented the fungi which were isolated from various samples that were taken at different sites. Aspergillus species was found very high intensity in these areas.

The bacterial species isolated from this research were many types like as *E. coli, Staphylococcus intermedius, Serratia marcescens, Micrococcus luteus, Pseudomonas putida, Streptomyces* species, *Proteus vulgaris,* and *Aeromonas caviae.* Studies revealed that Caselli *et al.*,[6] isolated *Bacillus subtilis, Bacillus pumilus,* and *Bacillus megaterium* during their research.

The fungal species isolated were *Aspergillus* species in various samples. This research agrees that many researchers reported that the genus *Aspergillus* was predominantly isolated fungus from biodeteriorated painted walls.

Mural painting from the different sites from Agra was investigated. The bacterial and fungal attacks were very high from the mural painting surface and the humidity values were also very high, especially in shady areas.

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