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A Study on Fungi Biodeterioration on Stone Monuments

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ABSTRACT: Fungi play a considerable role for the deterioration of cultural heritage. Due to their enormous activity. Fungal ability in production of organic acid has a crucial role in discoloration and degradation of different types of stone in culture heritages. Additionally, stone objects may support novel communities of microorganisms that are active in the biodeterioration process. In addition, microorganisms also colonize these monuments over a period of time, resulting in formation of biofilms; their metabolites lead to physical weakening and discoloration of stone eventually. This process, known as biodeterioration, leads to a significant loss of cultural heritage. This investigation focuses on mycological analyses of microbial biofilm from Pundarikakshan Perumal temple at Thiruvellarai, in Trichy District. In this work, the ability of fungi producing organic acids and biodiversity of fungal consortia was examined on the stone monuments.

Keywords: Biodeterioration, Fungi, Organic acid, Monuments, discoloration, Exopolysaccharide.

INTRODUCTION

The ecological group of rock-inhabiting fungus (RIF) is connected with terrestrial rocks. The persistent colonization of stones and peculiar geometrical parameters predicated on discoloration by slow growth endow microbes have impact in rock-inhabiting microbes and exotic potential in biotechnology, which helps to understand special status in ecology, and exotic potential of Microbial epilithic communities. The ability of fungi to produce organic acids and pigments plays a crucial role in the discolouration and degradation of different materials in cultural heritage objects. Acid production was found for the vast majority of strains tested, including all *Aspergillus* strains.

Fungal ability in production of pigments and organic acids have a crucial role in discoloration and degradation of different types of stone in cultural heritage objects (Miller *et al*., 2008). Additionally, stone objects may support novel communities of microorganisms that are active in the biodeterioration process. This investigation focuses on mycological analyses of microbial biofilm from two important buildings, made of granite and sandstone, and which were heavily colonized by fungi (Kusumi *et al*., 2013).

SAMPLING AND CULTURING

Sampling was carried out from three different sites of the monument located around Tiruchirappalli in 2022. Sterile needle was used for sampling and collected in a sterile zip lock cover and brought to the laboratory for isolation. Isolation performed followed by simple suspension of sample with 20 fold sterile water and plated on respective agar medium by pour plate method. Fungi isolated using Sabouraud Dextrose Agar (SDA) and Fungal spore and spore chain were observed under a light microscope.

SCREENING OF EPS AND ORGANIC ACID PRODUCING FUNGI

For the analysis and comparison of the biofilm-forming potential of each fungal monoculture, a standard qualitative biofilm assay, such as Congo red agar is used (Lan *et al*., 2010). This method is based on color changing of colonies on the Congo red agar (CRA) medium. Colonies with black color represent a biofilm producer whereas red-pink colonies retain non-biofilm producers. Detection of organic acid indicated by changes in pH using bromophenol blue (Ascaso *et al.*, 2002).

MODIFIED CONGO RED AGAR METHOD (CRA)

In the present study the modified Congo red agar (MCRA) was optimized to get strong black pigmentation at 48 hrs incubation and then for 2-4 days room temperature (Peeters.*et al*., 2008). Black colored colonies with dry crystalline consistency interpreted as positive biofilm producing strains. Red coloured colonies- interpreted as negative for biofilm production. (Composition of Congo red agar is peptone 5g; Sucrose 10g; Congo Red 0.4 g and agar 20 g water 1000ml). Congo red agar plate showing biofilm formation a) dry crystalline colonies-positive for biofilm and b) Red colored colonies-negative for biofilm (Sterflinger, 2010).

SCREENING OF FUNGI FOR SIGNIFICANT GROWTH AND PH REDUCTION

All fungal strains were incubated on SD medium (2g glucose, 1 g Sucrose, 1 g peptone pH 6.5 per L) to evaluate their organic acid production (Negi & Sarethy 2019). Fungal plugs were introduced and incubated for 5 days at 28°C (Carballal *et al.*, 2001). After incubation addition of Bromothymol blue is carried out to detect acid production.

ASSESSMENT OF EXOPOLYSACCHARIDE (EPS) PRODUCTION

Fungal strains are allowed to grow on modified Sabouraud Dextrose medium(Dextrose 40.000, sucrose 10 g and peptone 10 g/L) with congo red (Chiari & Cossio 2002). The cultures were then inoculated and incubated for 3 days at 28 °C. Production of EPS confirmed by development of black color colonies (Chiari & Cossio 2004).

RESULTS AND DISCUSSION

Colonies of blue green to gray with conidiophore were confirmed as *Aspergillus fumigatus,* Pale to brown colony with ascospore as *Fusarium* sp. olivaceous-brown with Conidiophore confirmed as *Cladosporium* sp, black color in the center and white edges as *Aspergillus niger.* wooly white was identified with *Alternaria*sp. green color colonies were identified as *Penicillium* sp.

Highly branched and aseptate hyphae were observed by *Aspergillus fumigatus, Fusarium* sp showed rarely branched, *Cladosporium* showed branched hyphae and *Aspergillus niger* showed well branched mycelium with segmented hyphae, *Alternaria* were observed by rarely branched septate hyphae and *Penicillium* sp also was observed by rarely branched but non septate hyphae. Color change was observed by *Aspergillus fumigatus, Fusarium* sp and *Penicillium* sp.

COLLECTED SAMPLE

1. Sample was collected in Pundarikakshan Perumal temple at Thiruvellarai at various at various sites; 2. Site II; 3. Site III

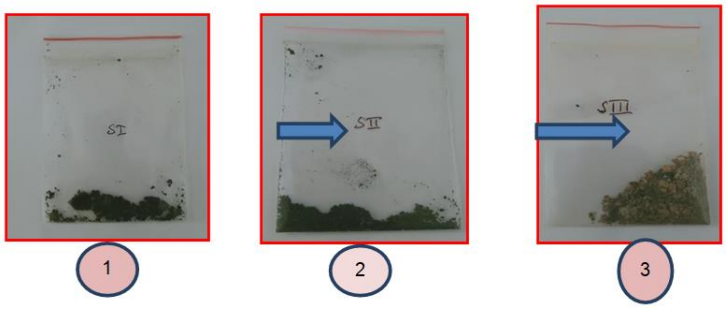
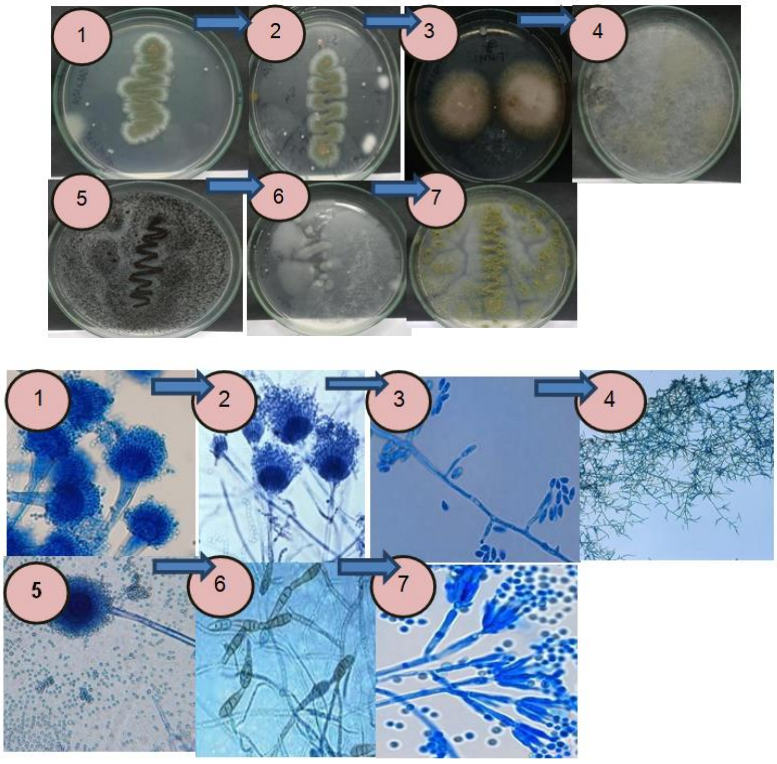


Fig. 1. Biodeteriorated sample from Perumal temple.

MICROSCOPIC AND MACROSCOPIC MORPHOLOGY OF ISOLATED FUNGI



1. *Aspergillus fumigates,* 2. *Aspergillus fumigates,* 3. *Fusarium* sp, 4. *Cladosporium* sp, 5. *Aspergillus niger,* 6. *Alternaria* sp, 7. *Penicillium* sp.

Fig. 2. Microscopic and macroscopic morphology of isolated fungi.

Table 1: Biofilm production on Congo Red Agar by Fungi.

Sr. No.	Name of the Fungi	Hyphae	Congo Red Agar	Organic acid
1.	*Aspergillus fumigatus*	Highly branched aseptate hyphae	-	-
2.	*Aspergillus fumigatus*	Rarely branched aseptate hyphae		
3.	*Fusarium* sp	Rarely branched aseptate	-	-
4.	*Cladosporium*	branched hyphae	-	+
5.	*Aspergillus niger*	Well branched Mycelium segmented hyphae	+	-
6.	*Alternaria*	Rarely branched, septate hyphae	+	-
7.	*Penicillium*	Rarely branched, aseptated	+	-

SCREENING OF EPS AND ORGANIC ACID PRODUCING FUNGI

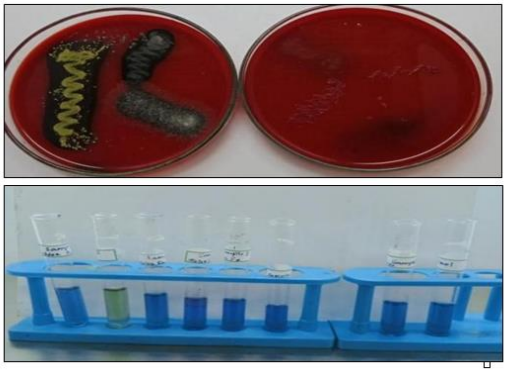


Fig. 3. Screening of EPS and Organic acid Production.

Out of seven tested fungi, isolates *Aspergillus niger*, *Alternaria* sp and *Penicillium* sp from sample 2 and 3 only show positive results on EPS production (Cuezva, 2012). Likewise the ability of organic acid using bromophenol blue indicates *Cladosporium* sp positive on organic acid production. Previously it was reported that application of a mixture of organic acids significantly promoted growth of pathogenic fungi, and increased the expression of chemotaxis-related gene (cheA) and biofilm formation (Cuzman *et al.*, 2011).

DISCUSSION

The maximum colony forming unit of sample 1 is 15×10^{-3} followed by sample three is 7×10^{-3} and minimum of 6×10^{-3} CFU in sample 2. The results of these studies vary substantially depending on the methods employed; this variability makes the study of bio deterioration of cultural relics complex and challenging. Culture-dependent methods are often considered less convenient than non-cultivation-based methods, which only enable the detection of 1–5% of the total microbial community (Dakal & Arora 2012).

CONCLUSIONS

Microorganisms play an important role in the biodeterioration of objects of cultural and historical significance, but their detailed biochemical and Eco physiological functions and roles remain unclear. Although many studies have reported that a high diversity of microorganisms participate in the biodeterioration process. Fungi is highly frequent in sample I. Isolates were identified based on cultural characteristics. Research on the biodeterioration of historical materials has revealed the presence of huge microbial diversity consisting of both biofilm producing and non-producing colonization. Few are organic acid producers and predominantly non organic aid producers.

FUTURE SCOPE

The production of organic acids and exopolysaccharides attracts scientific research attention due to its risk in causing destruction to stone monuments.

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Conflict of interest. None.

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