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Efficacy of fungal biodeterioration on stone monuments

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Abstract

Fungal ability in production of organic acid have crucial role in discoloration and degradation of different types of stone in cultural heritage objects. The aim of work was to study the Microfungi community on stone deteriorated Sample which were collected from Murugan Temple and Vinayagar Temple at Manapparai, both in Tamilnadu. Stone objects may support novel communities of microorganisms that are active in biodeterioration process. In this Research work, the ability of fungi producing Organic acids were analysed, Five fungi isolates were identified. Additionally, Growth rate of fungal isolates were observed in three different Enrichment medium (Glucose, Sucrose & Maltose as Carbon Source).The Wet and Dry weight of fungal mat was measured. The dry weight biomass was maximum in maltose enriched medium

Key words: ***Biodeterioration, Biomass, Dry and wet weight, Enrichment medium, Organic acids, Phenol red.***

Introduction

Biodeterioration – The word has only been in use for about 40 years. Hueck (2001), defined biodeterioration as "any undesirable change in the properties of a material caused by the vital activities of microorganisms". Fungi are especially concentrated on stone crusts. They are able to penetrate into the rock material by hyphal growth and by biocorrosive activity, due to the excretion of organic acids or by the oxidation of mineral forming cations, preferably iron and manganese. During the recent decades there has been a general concern about the deterioration of historic buildings. Some ancient masterpieces like, the Ajanta and Elora caves at Aurangabad, the Sun temple at Konark, the big temples at Thanjavur and Iravatheeswaraswamy temple at Darasuram which have been declared as world heritage monuments by UNESCO.

Microbiota of Monumental Rocks

A wide variety of biodeteriogens contribute to the deterioration of objects of archaeological significance. These organisms can cause direct or indirect damage to archaeological sites. Various microorganisms including bacteria, fungi, and lichens have been reported to cause biodeterioration of stone monuments. Cyanobacteria causes aesthetic damage to monuments by developing various colored microbial films on their surfaces. They enhance the local water retention capacity of the stone by dissolving the nearby material, which promotes further increase in their population.The Algal growth on stone surfaces is influenced by dampness, warmth, light and inorganic nutrients, particularly calcium and magnesium. Many algae show a marked sensitivity to pH on the surface, preferring acidic surfaces. But for some this value is not growth limiting. Algae can cause disfigurement and damage to stone monuments. Algae can cause direct as well as indirect damages by supporting growth of more corrosive biodeteriogens.

Fungi are believed to be potential contributors to decay of limestones, silicate minerals, iron and magnesium bearing minerals. The presence of organic residues on stone however encourages their growth. Fungi have ecological advantages over bacteria and algae as lithobionts due to their higher tolerance of low water potential, their effective modes of propagation and reproductive strategies and their ability to thrive even at poor nutrient concentrations (Hirsch *et al.*, 1995). Three different decay fungi types are classified and also found in buildings-brown rot, soft rot and white rot. Most of

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the brown rot and white rot fungi belong to Basidiomycetes, but some belong to Ascomycetes. Most of the soft rot fungi belong to Ascomycetes and Fungi imperfecti, e.g., *Chaetomium globosum* and *Phialophora hoffmannii* (viitanen *et al.*, 2000). Some of the genera commonly found in the stone deterioration are *Aspergillus elegans, Aspergillus flavus, Beauveria alba, Cladosporium* sp, *Cunnighamela echinulata, Curvularia* sp, *Fusarium roseum, Penicillium multicolour, Rhizopus arrhizus, Trichoderma* sp.

Materials and Methods

Sampling

The Study area selected for sampling was the Murugan temple and Vinayagar temple at Manapparai both in Tamilnadu. Visibly altered, discoloured and degraded sites on the walls were located. Wall scrapings were collected in each temple. The samples were stored at 4^{o}c for further processing.

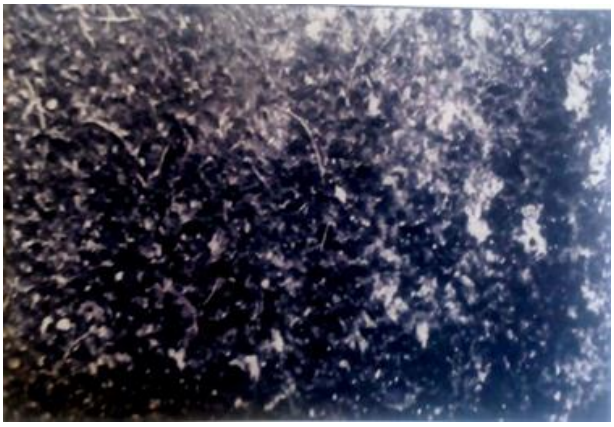

Figure1a. Sampling site of vinayagar Temple

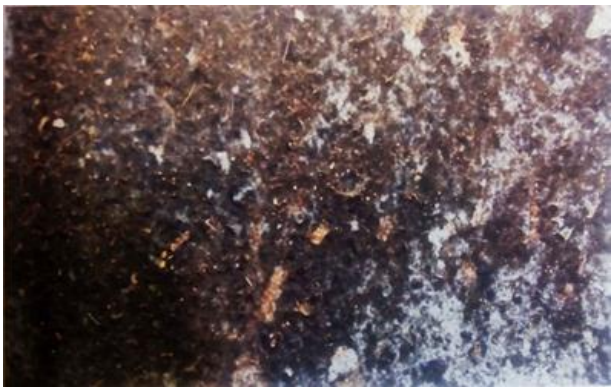

Figure 1b. Sampling site of Murugan Temple

Culture media and Inoculations:

Each sample was finely crushed into powder, from that composite sample 0.15 gram was placed in a sterile petriplate containing Mycological medium (Potato dextrose agar medium). The plates were then incubated at room temperature for 7 days. Macroscopic and microscopic characteristics of the obtained isolates were examined. Identification of fungi was based on the macroscopic features of colonies grown on agar plates, and the micro morphology of the reproductive structures was identified, top and reverse view on agar plate was identified.

Growth Rate of the Fungi

Solid media

The sterile media was poured into presterilized petriplates and was allowed to solidify. Then a loop-full of the fungi was inoculated at the centre of the plate and incubated at room temperature. The diameter of the colony was measured after 48 hrs of incubation for 7 days.

Liquid media

The Growth rate of the fungal isolates was observed in three different Enrichment medium with glucose, sucrose or maltose as the carbon source. 100 ml of sterile media was taken and it was sterilized, To this medium two loop full of fungal pure culture was inoculated and incubated at room temperature under shaking condition for 15 days. After 15 days, the fungal mat was carefully taken and placed on petridishes and dried at 80^{0}C for three daysm till the mat became dry. The wet and dry weight of fungal mat was measured.

Composition of the medium

Glucose / Maltose/Sucrose- 40g
Yeast Extract - 10g
Peptone - 0.5 g
Potassium di-phosphate ($KH_2 PO_4$) - 0.5 g
Magnesium sulphate ($MgSO_4$. $7H_2O$- 0.5 g
Distilled water - 1000 ml

Screening For Organic Acid Produced By Fungi

Screening of organic acid production by fungi was done by using phenol red as pH indicator. Phenol red was added @ 0.025 g/litre to potato dextrose agar medium. Fungal isolates were inoculated, to the respective plates. The plates were incubated for 3-4 days at room temperature. Control plates were maintained. After incubation the change in media colour from red to yellow indicated positive for organic acid production.

Result and Discussion

Macroscopic morphology of fungal isolates

- Isolate I was a rapidly growing white coloured fungus which swarmed over the entire plate. It showed cottony and fuzzy growth which gradually changed to grey. The reverse was pale white in colour.
- Isolate II was also a rapidly growing organism which matured within 4-5 days. Showed a cottony white growth. The reverse of the colony was pale yellow in colour.
- Wooly, white, fuzzy colonies which gradually changed to pink was observed in isolate III. The reverse side of the colony showed a central orange area and yellow coloured periphery.
- The fungal colony of Isolate IV was initially white and eventually turned black from front and reverse. The appearance of spores took around 10-12 days.
- The fungal colony of Isolate V was flat, velvety in texture initially white in colour and gradually changed to yellow. The reverse was dark brown
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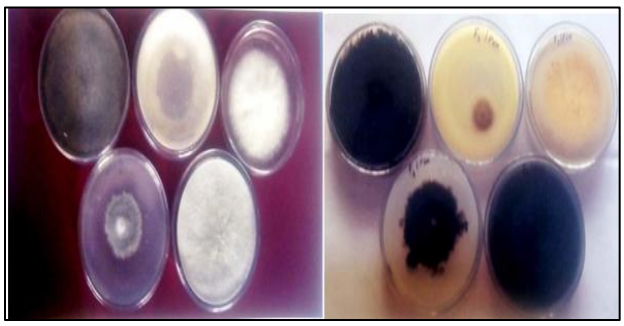

Figure 2. Top and Reverse view of Fungal Isolates

Microscopic Morphology of fungal isolates

Isolate I showed on septate and broad hyphae which gave rise to single sporangiophores with globural sporangium. Sporangiophores were short and single. Non septate, broad hyphae were observed on the lactophenol cotton blue mount of Isolate II. Sporangiophores were erect and form short lateral branches each of which terminates in a swollen vesicle. The vesicle has spine-like denticles on its surface. Sporangioles are round to oval in shape. Isolate III had multi-celled crescent shaped macroconodia. Conidiophores arised from septate hyphae septate hyaline hyphae and coniodiophores were observed on the wet mount of Isolate IV. Conidia black, globose, one-celled, borne on a flattened, colourless vesicle at the end of a coniodiophore Isolate V showed a septate hyaline hyphae Coniodiophores carry the phialides at their tips.They are usually grouped in pairs or brush-like clusters yellowish brown, elliptical microconidia.

From the above observations the fungal isolates were identified as,

- Isolate I - *Mucor* sp
- Isolate II - *Cunninghamela* sp
- Isolate III - *Fusarium* sp
- Isolate IV - *Nigrospora sphaerica*
- Isolate V - *Paecilomyces* sp

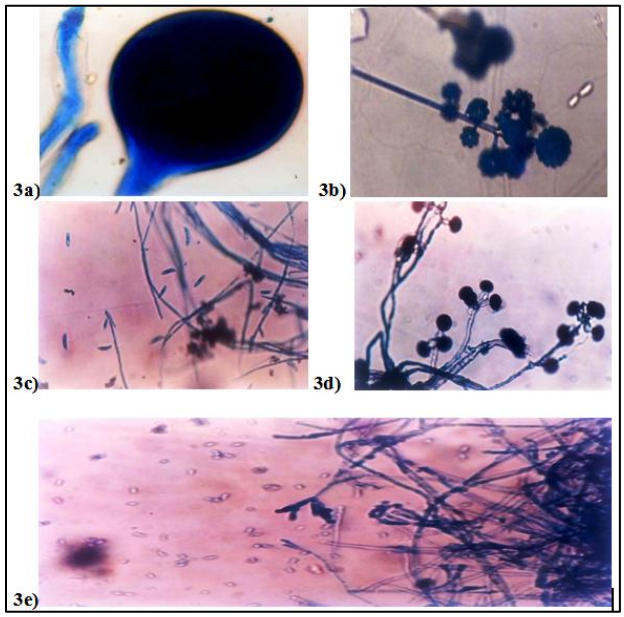

Figure 3. Isolates of Micromycetes (3a: *Mucor* Sp, 3b: *Cunnighamela* Sp, 3c: *Fusarium* Sp, 3d: *Nigrospora sphaerica* , 3e: *Peacilomyces* Sp)

Growth rate on fungi on solid media

After 48 hours of incubation the diameter of the colonies were measured for seven days. Growth was seen in the first three isolates after 48 hrs and isolate IV and V visible colonies appeared after four days of incubation. As per the literature available (Benson and Cappuccino) *Mucor*, *Cunninghamela* and *Fusarium* sp. are fast growing fungi. Growth rate was generally faster on potato dextrose agar. Growth rate on liquid media was determined by measuring the dry weight biomass of the fungal genera. The growth was observed in three different enrichment media having different carbon sources. The dry weight biomass was

Table 1. Growth Rate of Fungi on Potato Dextrose Agar and Rose Bengal Agar Plate Growth rate on liquid media

DAYS After 48 Hours Incubation	Diameter of the Colony (cm)									
	Mucor sp		*Cunnighamela* sp		*Fusarium* sp		*Nigrospora spherica*		*Paecilomyces* sp	
	PDA	RBA	PDA	RBA	PDA	RBA	PDA	RBA	PDA	RBA
1	4.6	1.8	6	3.8	0.2	0.2	1.6	-	0.6	-
2	8.8	3.0	8.8	7.6	2.0	1.4	2.4	0.8	1.8	1.0
3	9.0	4.6	9.0	8.0	4.0	3.0	7.0	4.0	5.0	5.0
4	9.0	7.0	9.0	9.0	7.0	7.8	9.0	6.2	6.8	6.0
.5	9.0	9.0	9.0	9.0	9.0	8.2	9.0	8.0	8.8	9.0
6	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
7	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0

Figure 4: Growth on Enrichment medium.4a: Glucose; 4b: Sucrose; 4c: Maltose

Figure 5. Screening for the Production of Organic acid of Fungal isolates

maximum in maltose enriched medium for *Mucor*, Cunninghamela and *Nigrospora sphaerica*. The growth of the fungi in the entire carbohydrate supplemented medium supports the fact that these organisms produce extracellular polyssaccharides for their growth and survivability on the walls.

Screening for organic acid production

Color change was observed in medium incorporated with phenol red. This confirms that all fungal and isolates produced organic acid except fungal isolate II (*Cunninghamela* sp.). This confirms that the microbial fungal strains isolated might cause deterioration of stones on account of organic acid production.

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