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A STUDY ON IDENTIFICATION OF STONE TEMPLE COLONIZING COMMON FUNGAL ISOLATES

Dr. R. Kalaivani*, G. Nandhini & G. Keerthiga****

* Assistant Professor, PG & Research Department of Botany and Biotechnology, Bon Secours College for Women, Thanjavur, Tamilnadu

** PG & Research Department of Botany and Biotechnology. Bon Secours College for Women, Thanjavur, Tamilnadu

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Abstract:

Stone cultural heritage are at risk of bio-deterioration caused by the diverse population of living micro organisms as biofilms. The microbial metabolites of bio films are responsible for deterioration of under laying substratum and may lead to the physical weakening and discoloration of wall especially the fungal colonies producing pigments and organic acid which have crucial role in the discoloration and degradation of cultural heritage object. The fungal species are isolated from the wall of the temple, AIYARAAPAR at Thiruvaiyaru in Thanjavur district. Temperature, light intensity, air, humidity and concentration of No_2 and Co_2 are the factors influencing the fungal growth. This present study is indented to isolate and identify the temple colonizing common fungal species. This fungal isolate causes air pollution and deterioration. In turn we may use microbial consortium for remediation of biomass. Microbial biotechnology plays a vital role in providing harmless mode of renewal on our monuments. In this present study, mold, Aspergillus flavus, Aspergillus niger, Penicillium sp., Cunningamella sp., were isolated.

Key Words: Bio-Deterioration, Bio Film, Discoloration & Remediation

1. Introduction:

Historical monuments are the pride for every nation. They are not only considered as our cultural heritage but also the learning resources of science and technology. A large percentage of the world's tangible historical monuments are made from stone, and it is slowly but irreversibly disappearing. The transformation of stone into sand and soil is a natural recycling process, essential to sustain life on earth. The deterioration of stone monuments represents an eternal loss of our cultural heritage. The most common stone monuments are constructed by marble and limestone, of the calcareous type, sandstone (which is mainly quartz, feldspar, and iron oxide) and granite (mainly quartz and feldspar), of siliceous type.

These differ in hardness, porosity, and alkalinity, properties that affect their susceptibility to biodeterioration. These stone types are not discrete. In addition, the materials frequently used to stabilize the building blocks and to coat the surface prior to painting could be considered. These are human-made hence vary in composition and levels of organic materials. They are extremely susceptible to biodeterioration, as is the modern stone substitute, concrete. The microflora of external stone surfaces represents a complex ecosystem, which includes not only algae, bacteria, fungi, and lichens, but also protozoa; in addition, small animals, such as mites, may also be present. Stone inhabiting microorganisms may grow on the surface (epilithic), in more protected habitats such as crevices and fissures (chasmolithic), or may penetrate some millimetres or even centimetres into the rock pore system (endolithic) (Tiano, 2002).

Biodeterioration processes are rarely caused by one distinct group of microorganisms, but are rather an interaction of coexisting groups. Among the microbial consortium, fungi play major role in biodeterioration. The highest degree of biodeterioration occurs in the tropics, because of high humidity and temperatures. The stone fungal colonies is considered to be very aggressive, with a high capacity for ''biocorrosion'' (more properly called ''bioerosion'') and biofouling (Warscheid, 2003). These two terms are defined by Warscheid (2003) as: (1) microbially induced or influenced corrosion of materials, altering the structure and stability of the substrate, and (2) the presence of colloidal microbial biofilms on or inside materials, leading to visual impairment and potentially altering the physiochemical characteristics of the substrate. In recent years, molecular methods have been developed for identification and enumeration of microorganisms in environmental samples. (Amann *et al*., 1995).

Figure 1: Sample collection site- AIYARAAPAR temple

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The temple, AIYARAAPAR is located in Tiruvaiyaru, a panchayat town in Thanjavur district in the Indian state of Tamil Nadu (Fig.1). It is situated on the banks of the river Kaveri, 13 km from Thanjavur, Thiruvaaiyaru has an old Shiva temple dedicated to Panchanatheeswar. Though pilgrims flock to this temple throughout the year, Thiruvaiyaru is more renowned for its association with Saint Thyagaraja, who, along with Muthuswami Dikshitar and Shyama Sastri, comprises the Trinity of Carnatic music.

Several inscriptions in the temple affiliates the temple to the Cholas, Pandyas, and other rulers. Karikala Chola, Rajaraja the great, Jatavarman Sundara Pandyan, and Krishna Devarayar are associated with Thiruvaiyaru. The temple has two distinct divisions called 'Uttarakailasam' and 'Dakshinakailasam'. Uttarakailasam was built by Rajaraja Cholan's queen in the late 10th century who also made several endowments. Dakshinakailasam was renovated by Rajendra Cholan's queen. Appar, one of the important Nayanmar, was closely associated with this shrine and dedicated one of the songs in 'Thevaram' to this temple (Census of India, 1961). This present study is intended to isolate and identify the common temple colonising fungal species from AIYARAAPAR temple wall at Thiruvaiyaru in Thanjavur district.

2. Materials and Methods:

2.1 Sample Collection: The sample was collected from AIYARAAPAR temple wall at Thiruvaiyaru in Thanjavur district. The samples were then taken to the laboratory using sterilized cellophane bags.

2.2 Isolation of Fungi from the Soil Samples (Warcup, 1950): The serial dilution and plate method on media such as Potato Dextrose Agar was used for isolation techniques. 1g of sample in 10ml of sterile distilled water. Dilutions of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid overcrowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Petri dishes, in triplicates of each dilution, containing sterile Potato Dextrose Agar medium. 1% streptomycin solution was added to the medium for preventing bacterial growth, before pouring into Petri plates. The plates were then incubated at 28+2^{o}C for 4-7 days. Organisms were easily isolated because they formed surface colonies that were well dispersed, particularly at higher dilutions.

2.3 Inoculating Techniques: The working benches in the laboratory were thoroughly swapped with methylated spirit soaked in cotton wool, and also a burning blue flame was allowed to sterilize the surrounding air before the inoculation proper. The conical flasks were corked tightly with cotton wool and the Petri dishes were fully autoclaved.

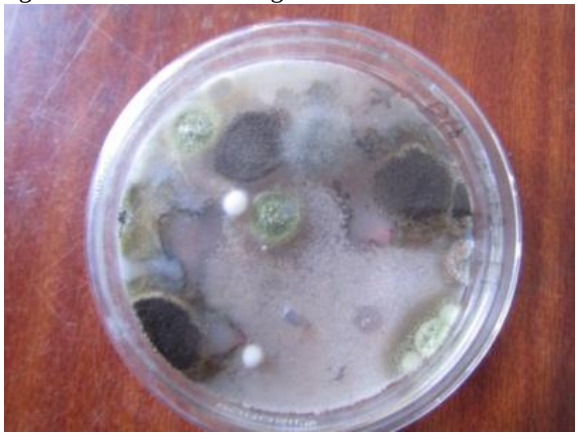
2.4 Identification of the Soil Fungi (Diba et al., 2007): Generally identification of the fungal species is based on morphological characteristics of the colony and microscopic examinations. The colony growth which includes length and width of the colony, the presence or absence of aerial mycelium, the color, wrinkles furrows and any other pigment production were the macro morphological characters evaluated.

2.5 Staining Technique for Fungi: Inoculating needles were flamed over the burning Bunsen burner. Then using the needle, a small portion of the growth on the culture plate was transferred into the drop of lacto phenol in cotton blue on the slide. The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium. The specimen is observed under the microscope for microscopic identification.

3. Results and Discussion:

The fungal composition of the samples from temple wall had ten predominant species. *Rhizopus stolonifer, Trichoderma harzianum, T.viride, A. niger, A. tamarii, A. nidulans, A. Flavus, Penicillium, Cunningamella sp.,* and *mold.* The four predominant fungal genera i.e., *Aspergillus species were present* in the samples which represented 40% of the fungal communities. 20% of *Trichoderma species* were found in wall sample. The fungal species like *Rhizopus stolonifer, Penicillium, Cunningamella sp.,* and *mold* were present in 10% respectively. The results were showed in Fig. 2 & 3.

Figure 2: Isolation of Fungal colonies in PDA Medium



The colonies of *Aspergillus flavus* were present as velvetting in nature yellow to green. The conidiophores are of variable length rough pitted and spiny the sterigmata are made of two in rows in phialides which cover the entire vesicle. The hyphae were septate. The *Aspergillus niger* colonies are usually at first white yellow then turning to dark black. The conidiophore is of variable length sterigmata are double cover entire vesicle from radiate head. The vesicle is double layered. Yellow green to olive brown colour, phialidas usually biseriate, sometimes uniseriate, conidiophore is rough was represented as *Aspergillus tamari.*

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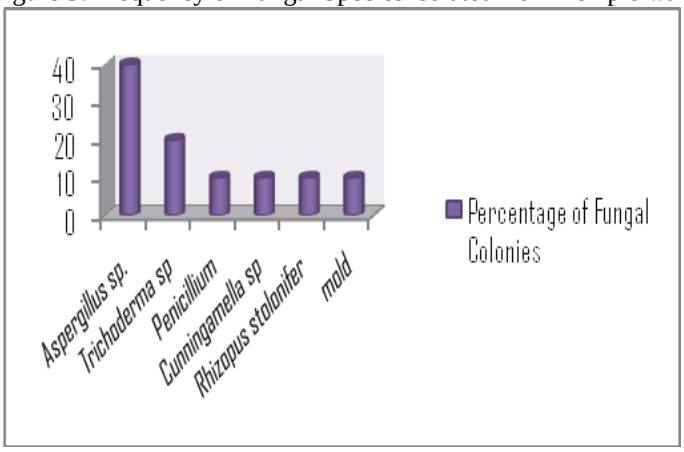
The *Penicillium* showed moderately producing a folded colony which becomes concerned with floccose grayish mycelium. *Penicillium sps* arranged in an asymmetric series. It showed highly branched septate hyphae. The hyphae were multibranched. It gave a brush like appearance spheroid green conidia on asymmetrical phialids which were unicellular. The growth was rapid and produced aerial myceta. The colonies were soft, white colour at first and later it became white to grey and flash at the stage of sporulations and without rhizoids.

Figure 3: Frequency of Fungal Species isolated from Temple wall



Fungi are the major decomposers of dead organic matter and contribute significantly in recycling of nutrients in natural and modified ecosystems. Altogether five samples from five different locations were examined for fungal diversity. The study resulted the presence of 10 species of fungi were identified and characterized from PDA plates (Table: 1).

Table 1: Identification of Fungal species from the temple wall

S.No	Identified Fungal species from the sample
1	*Rhizopus stolonifer*
2	*Trichoderma harzianum*
3	*T.viride,*
4	*A. niger,*
5	*A. tamarii,*
6	*A. nidulans,*
7	*A. Flavus,*
8	*Penicillium,*
9	*Cunningamella sp.,*
10	*mold.*

PDA medium is the most commonly used culture media and was stated to be the best media for mycelia growth by several workers worked with it earlier [Maheshwari *et al*.,1999; Saha *et al*.,2008] due to its simple formulation and potential support to wide range of fungal growth. Characterization of the isolates up to genus level and to the species level was made based on the macro morphological and micro-morphological characters by using authentic manuals of soil fungi.

Monuments that have survived thousands of years as relicts of extinct cultures have experienced accelerated aging in recent years (Gaylarde and Morton, 2002). Distant from the microorganisms present in the immediate environment, many factors influence the deterioration of stone monuments. Physical, chemical, and biological agents are associated and act on stone monuments. The physical properties of the stone influence the extent of degradation. For microbial growth, rough surfaces and high porosity favor adhesion and colonization. (Caneva *et al.*, 1991; Warscheid and Braams, 2000; May et al (2003).

Environmental pollution, which has increased rapidly within the last century, may influence stone degradation directly (e.g., acid rain) or indirectly, by supplying nutrients for microbial growth. It has been shown to enhance detrimental microbial activity on the stone substrate (Sand *et al*., 2002; Herrera and Videla, 2004).

Microbial cells may contribute directly to the deterioration of stone by using it as a substrate or indirectly by imposing Biodeterioration of Stone. Physical stress, serving as nutrients for other organisms, or providing compounds for secondary chemical reactions (Sand, 1996). Surface biofilms are microbial cells embedded in extracellular polymeric substances (EPS). The simple presence of a biofilm has aesthetic, chemical, and physical effects on the stone. Discoloration is mainly an aesthetic problem (Fig.4).

It may be caused by pigments released from, or contained within, the microorganisms (melanins, carotenes, and photosynthetic pigments). Physical damage may be caused by penetration of filamentous microorganisms (particularly fungal hyphae) into the stone (Hirsch *et al*., 1995a). Meristematic fungi produce swollen, isodiameteric cells with thick, melanin containing cell walls. They remain metabolically active even in low nutrient conditions and have high resistance to desiccation, UV radiation, and osmotic stress, thus being well adapted to growth on external walls. Wollenzien et al. (1995) suggested that these are the resident fungi in Mediterranean climates; the fast growing, filamentous hyphomycetes being present only in the colder and more humid winter months and therefore considered contamination in this climatic area.

Figure 4: Discoloration of stone heritage at AIYARAAPAR temple

Resende et al. (1996) identified a wide range of filamentous fungi in soapstone and quartzite in churches in the Brazilian state of Minas Gerais. The most common genera were *Cladosporium* and *Penicillium*. However, it must be emphasized that the detection technique affects the results of such investigations. Gorbushina et al. (2002) detected mainly deuteromycetes, such as *Alternaria*, *Cladosporium*, and *Trichoderma*, on historic marble monuments in St. Petersburg and Moscow. Sterflinger (2000) indicated *Aspergillus niger*, *Penicillium simplissimum*, and *Scopulariopsis brevicaulis* were as important fungi that attack siliceous stone. These dark pigmented mitosporic fungi can actively penetrate limestone and marble. They produce pits of up to 2 cm diameter on rock surfaces. They are especially important in arid and semiarid environments because of their ability to resist high temperatures, desiccation, and osmotic stress.

In fact, several cryptoendolithic fungi may actively bore into the stone and hence physically disrupt its integrity (Hoffland *et al*., 2004; Gadd, 2007). As fungi, do not require light for growth, their boring activity can penetrate to greater depths. It can form cracks, fissures, crevices, and lead to the detachment of crystals.The removal of the microbial community from any given stone monuments surface is an intervention that must be carefully evaluated. Biocidal treatments may have negative effects on the artifacts (Webster *et al*., 1992). The removal of the microbial community may give rise to a new succession of microorganisms, which may be more damaging than the old microbial surface populations; and the inhibition of specific groups of microorganisms may favor the growth of others. The approach to control biodeterioration must be a multifaceted, interdisciplinary one that considers the history and condition of the art as well as physical and chemical damaging factors.

Thus the biotechnological approaches include application of self healing bacterial cement at the site of damage, may pave a way to achieve sustainable mode of conservation and preservation. The carbonatogenesis microbial approach imply reduction of green house gases effect which lead to global warming, the great havoc.

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