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Study of Fungal Flora on Deteriorating Sandstone Structures of Historical Monuments

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ABSTRACT

Sandstone monument may support biocommunities that are active in process of deterioration. The bio-film made on sandstone consists of a complex of bryophytes and fungi consortia. Hyphae, filaments and spores form fungal components while bryophytes form the photosynthetic part of the bio-film. These structures interconnect on the surface of sandstone monuments to form a narrow layer. In the present investigation 11 samples were collected from different sites which are made up of sandstone. 6 biocommunities, which is dominated by the sandstone structures of the monuments. During the time of the investigation, it was clear that *Aspergillus sp.* was most dominant followed by *Rhizopus sp*. The recognized biocommunities cause mechanical exfoliation of building as well as stone material discoloration that was evaluate through production of dark pigments and mechanical hyphae penetration and organic acids.

***Key words*:** Degradation, Deterioration, Sandstone monument, Biocommunities, Bio-film

India is amalgamation of different cultures from north to South and from East to West. The cultural effects can easily be seen in the colors and shades of monuments, temples, mosque, forts, palace, mausoleums, cave etc. that would be built by different emperors for different reasons. These are our incredible, immortal magnificent cultural heritage. A survey of the art and architecture of the Indian subcontinent, indicate the stones chosen for building, sculpture and ornamentation, would be often associated with prominent geographic and geological features of the local area. The scraps of Vindhya Mountain are the prominent features of the central Indian states. These hills comprise of sandstone and quartzite that provided the structural and sculptural materials used through to these days for building [1]. There are three types of rock stones i.e., igneous rocks, sedimentary rocks and metamorphic rocks, which are used in building construction. However, this study is based on sandstone constructions.

Sandstone is a sedimentary rock. Sedimentary rocks are initially deposited in an incoherent form as sediments. These sediments are eventually combined by the deposition of cementing matter stratified rocks and by pressure from the weight of overlaying sediments. Sandstone is made up of mainly quartz, feldspar and lithic fragments. Other minerals also take place, determined by the mineralogical maturity of the stone, it is these minerals that make study of provenience possible in the study of stones.

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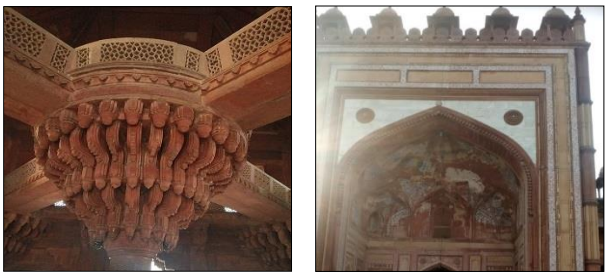

Fig 1 Fatehpur sikri

In India followings varieties of sandstone are use Kandla Gray (Bhilwara gray), Rajpura green (Bhilwara green) Marson copper (Bhilwara brown), chocolate, Gwalior greenish white (GWT-MINT), Lalitpur yellow (LLP-YELLOW), Dholpur beige, Dholpur pink, Agra red (Dholpur red), Khatu teak and Khatu rainbow. In all of these varieties, Dholpur being is buff white colored and highly used variety. It has been used from over centuries and is a constituent of a large number of cultural heritages. The deterioration of all the sandstones generally includes the separation of different layers leading, at times, to splitting of stone pitting and powdering of the surface.

Fig 2 Orchha temple and Mariam tomb

In sandstone with calcite and dolomite as cementing material though has good cementing properties, is readily attacked by carbon -di-oxide and other acidic gases present in the air or dissolved in the water. The dissolution of a relatively small amount of cementing material loosens a relatively large number of quartz grains and thus they are weathered easily. Similarly, the sandstone that have clay as binding medium are the poorest with regards to resistance to weathering. Because clay has no good cementing properties and when the stone is wetted by rain or dampness [2]. Sandstone surfaces are repeatedly exposed to biological, chemical and physical degradation. The growth of fungal on object of cultural heritage most of the time causes a major aesthetical spoiling due to colonization and pigmentation on fungal. Moreover, fungi degrade sandstones and thus influence object substantially. The enzymatic degradation of organic cause loss of paint layers or even reduction. Fungi migrate and penetrate underneath layers of paint thus detachment [3]. The fungal flora in museums is also particularly affected by the carbonate, minerals and other carrying atmospheres [4].

Microbial metabolites of bio-films are responsible for the deterioration of elemental structure and can lead to physical weakness and discoloration of stones. The status of cultural heritage, depending on their use, also plays an important role, which spoils cultural monuments. The progress of definite sp. on the surface of a particular sandstone is determined by the properties and nature of the sandstone. The counteracting of a potentially colonized surface organism depends on ecological species that include biochemistry that actively participate in weathering of minerals. Microbial processes that lead to mineral degradation can include microbial depletion and oxidation, maintenance and, production of acidic metabolites and the creation of appropriate physicochemical conditions. These microorganism-mediated processes are relatively responsible for the physical and chemical weathering of rocks, which ultimately, lead to soil emergence. Biocommunities can also provide for the decline of stone artifacts such as historical monuments and statues.

Aspergillus is the most valuable species for biotechnology in section *nigri* and its species is of wide occurrence. Some biocommunities recognized as *Aspergillus costarincaensis* which isolated from soil in Costa Rica and produces large pink to greyish brown sclerotia. Seth *et al.* [5] were recorded 8 species of *Aspergillus* viz. *Aspergillus luchuensis, Aspergillus flavus, Aspergillus oryzea, Aspergillus niger, Aspergillus terreus, Aspergillus variecolor, Aspergillus awamori, Aspergillus niveus.* The aim of this research is to study the biocommunity on sandstone monuments by using microscope observations and molecular technique in order to evaluate the importance value index and damage caused by fungi.

MATERIALS AND METHODS

The present paper deals with various methods used in analysis of biodeterioration of sandstone monument. The methodologies involve the identification of fungi. Samples of sandstone were collected from eleven localities: Red Fort (Agra), Akbar Tomb (Agra), Fatehpur Sikri (Fatehpur), Mariam Tomb (Agra), Etma Ud Daula (Agra), St. Johns (Agra), Kailash Temple (Agra), 64 Khamba (Agra), Ochha Temple (Jhansi), Khas Mahal (Fatehpur Sikri) and some unidentified monuments. Under the observation visible degradation and alteration were mapped and after that the samples were collected. Sandstone sample were taken for myco- logical analyses by swabbing surfaces with sterile cotton swabs. The samples were then stored at 4°C.

Isolation of microflora

The samples were collected with the help of sterilized tools (scalpes, rushes, swab and cellophane tape) these are preserved at 4°C until the time of analysis in the laboratory. In the present study isolated was performed directly from the monuments and from collected deterioration sandstone samples.

Historical monument

Scrapping method: The area sampled exhibited black and Brown sports distributed on sandstone surface. These samples were taken from the surface of the stone using sterile scalpels and lancets and scrapping of the surface material to a depth of 1-3 mm, and then transported to the laboratory in sterile vials.

Cellophane tape method: The sampling of fungal growth directly from the affected sandstone wall with the help of stickly tape. The sticky tape directly removes the powdered stone together with fungul fruiting bodies. In this way, direct identification of fungi becomes much easier. These samples were then cultured in the laboratory for further investigation with the help of microscope.

Swabbing and serial dilution method: In this method the surface of deteriorated sandstone sample was swabbed by sterilized moist cotton and shaken in 10 ml of sterilized distilled water. Serial dilutions 10^{-2},10^{-3}……10^{-7} were made by pipetting measured volumes (1ml) into additional dilution blanks (having 9ml sterile water). Finally, 1 ml aliquots of various dilution were added 20 ml of the sterile, cool molten (45°C) media (Czapeck-dox agar/ rose Bengal agar for fungi and Nutrient agar for bacteria). The dilution 10^{-2} to 10^{-5} were selected for enumeration of fungi and 10^{-4} to 10^{-7} for bacteria. Upon solidification, the plate was incubated at 25°C for fungi and 35±1°C (for bacteria) for 3-7 days and 24 -72 hours respectively.

The convenient techniques used for bacteria and fungi were applicable to microscopic algae too. Only with the difference incubation conditions, 30-35°C temperature, light of 60W tungsten, 15-20 days and grown in Beneck's broth priegsheim and modified Knop's broth.

Molecular and morphological identification of fungus

DNA isolation, PCR using universal primers for the type of organism, purification of the PCR amplicons, cycle sequencing reactions, purification and run them on an automated capillary-based Sanger DNA Sequencing system. At every step, there is in-house quality check to ensure

success of the sequencing reactions. Post sequencing, fragments are manually checked and only good quality seqeunces are used to form contigs, which are then matched in well-curated databases for assigning closest neighbor as the tentative identification of organism.

Calculations

Various myco-ecological parameters have been calculated using the following formulae:

$$\text{Frequency (F\%)} = \frac{\text{No. of plates in which specific organism occurred}}{\text{Total No. of plates examined}} \times 100$$

$$\text{Density (D)} = \frac{\text{Total No. of colonies of specific organism}}{\text{Total No. of plates examined}} \times 100$$

$$\text{Abundance (Ab)} = \frac{\text{Total No. of colonies of specific organism}}{\text{No. of plates in which organism occurred}} \times 100$$

RESULTS AND DISCUSSION

During screening for search of biocommunities, total six species of Microorganisms were isolated from different sandstone monuments. In these monuments *Aspergillus constaricaensis* shows maximum frequency, Density as well as Abundance followed by *Rhizopus sexualis*. Some of the fungal species are confined to particular area i.e., *Rhizopus oryzae*, *Aspergillus luchuensis*, *Aspergillus aflatoxiformans*, *Rhizopus americanus*. These confinements of microorganism depend on environmental conditions of the area, which fluctuates from geographical area to area [6]. In the present study *Aspergillus sp.* are the most widespread sp. establish in the sites. The biocommunities deterioration of sandstone monuments from the Osirion's Sarcophagus Chamber as infected by increasing ground water level was detected by the following fungi; *Cladosporium cladosporioides*, *Aspergillus terreus*, *Curvularia lunata*, and *Acremonium falcifrome*. The results comprise embellishment by chemical weathering organic substances, and formation of crusts, encourage the colonization of natural sandstone by fungi and thus increase the processes of biodeterioration [7]. The deterioration of sandstone there is equivalent contribution by biocommunities and the structure of sandstone. Therefore, the nature of the substrate, the relationship between the substrate and the organism, the relationship between the design and development of the organism, their distribution of frequency are important components in stone monument protection interventions. Abdelhafez *et al.* [8] recognized to belong to the follow 8 genus which are *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Stachybotrys*, *Alternaria*, *Acremonium*, *and Cladosporium.*

Table 1 Fungal isolates

Isolated fungi	Culture plates											F%	D	Ab
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}			
Aspergillus constaricaensis	1	2	1	1	3	-	-	-	-	-	-	0.0045	0.72	1.6
Aspergillus luchuensis	-	-	1	-	2	-	-	-	-	-	-	0.0018	0.27	1.5
Aspergillus aflatoxiformans	-	-	-	-	-	4	1	-	-	-	-	0.0018	0.45	2.5
Rhizopus sexualis	-	-	-	-	-	-	-	2	3	-	1	0.0027	0.54	2
Rhizopus americanus	-	-	-	-	-	-	-	2	4	-	3	0.0027	0.81	3
Rhizopus oryzae	-	-	-	-	-	-	-	-	-	2	-	0.0009	0.18	2

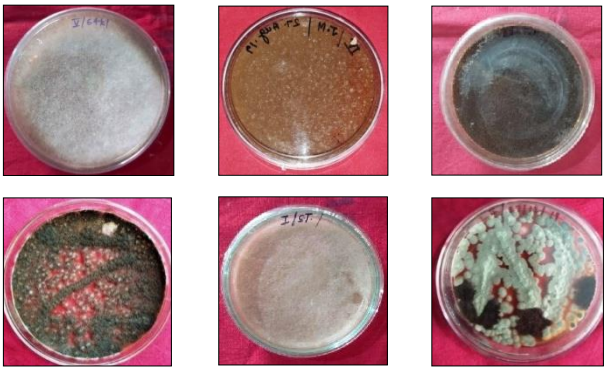

Fig 3

Variation in the composition of these biocommunities depends on the current environmental conditions of the host and the biochemical nature, the degree of competition among fungal organisms. The relative frequency (R.F.) and frequency (F.) are indirectly or directly connected with climatic conditions and meteorological data [9]. In each fungal biocommunity all the sp. is not equally important there are relatively only few of these sp., which decide the environment of the community [10]. These few biocommunities apply a most important predominating impact on the community and play fundamental role in deterioration of various substrates. The most important biodeteriogens of the historical monuments and artworks [11]. Experiments in laboratory have verified that basic rocks are more vulnerable to attack on fungal than acidic rocks. *Aspergillus Niger* left some metal ions from the rock samples [12]. Li *et al.* [13] showed in that urease-positive fungus may take part in major role in environmental fate, bioremediation or biorecovery of Sr or other metals and bionuclides that form insoluble carbonates. Some studies have shown that the classification structure of an epilithic biofilm by molecular technique, which is the terminal restriction fragment length polymorphism and amplicons pyriding [14].

Identification

Sample were collected from different monuments about 11 fungus were isolated, purified and identified as, with *Aspergillus costaricaensis*, *Aspergillus luchuensis* *Aspergillus aflatoxiformans* isolate DTO 228-G2, *Rhizopus sexualis* var. *americanus*, *Rhizopus oryzae* CBS 111.07 was identified as a close relative to *Aspergillus*. The identification test for 11 most common fungus sp. Is given in (Table 2). These results are similar to those reported by Jedidi *et al.* [15] who stated that *Aspergillus flavus* was isolated in corn. The

occurrence of *Aspergillus flavus* is consider very important because they are recognized to produce aflatoxins which are considered the most powerful carcinogenic to human being and natural world.

Table 2 Identification of Fungus

PRN	Strain No.	Closest neighbor	Accession No.	% Similarity
A_MAR_19_112	M.T.1	*Aspergillus costaricaensis* CBS 115574	MW001760	98.60
A_MAR_19_113	F.S.2	*Aspergillus costaricaensis* CBS 115574	MW001761	98.16
A_MAR_19_114	A.T.3	*Aspergillus costaricaensis* CBS 115574	MH862988.1	99.07
		Aspergillus luchuensis KACC 46772	MW001762	99.07
A_MAR_19_115	E.4	*Aspergillus costaricaensis* CBS 115574	MH862988.1	98.64
A_MAR_19_116	R.F.5	*Aspergillus costaricaensis* CBS 115574	MH862988.1	99.22
		Aspergillus luchuensis KACC 46772	MW001763	99.22
	I-M.T.	*Rhizopus oryzae* CBS 112.07	MT913006	99.26
C_SEP_19_009	II-K.T.	*Aspergillus aflatoxiformans* isolate DTO 228-G2	MW001764	99.80
C_SEP_19_010	III-O.T.	*Rhizopus sexualis* var. *americanus*	MW001765	99.07
C_SEP_19_011	IV-J.M.	*Rhizopus sexualis* var. *americanus*	MW001766	99.78
C_SEP_19_012	V-61K	*Rhizopus oryzae* CBS 112.07	MW001767	99.19
C_SEP_19_013	VI-KH.M	*Rhizopus sexualis* var. *americanus*	MW001768	99.78

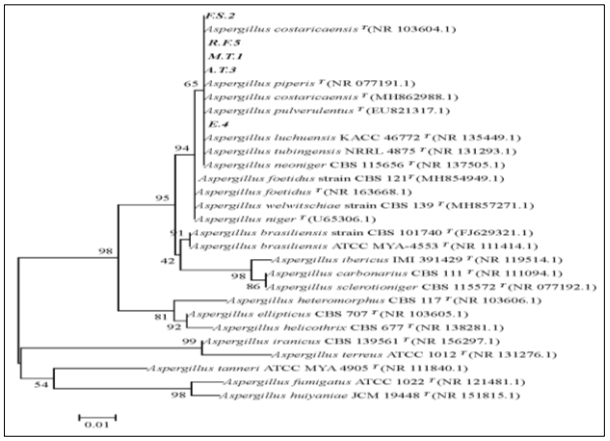

Fig 4 Report ID: A_MAR_19_112_116 Strain Details: Strain 1: F.S.2, R.F.5, M.T.1, A.T.3, E.4 Phylogenetic Tree

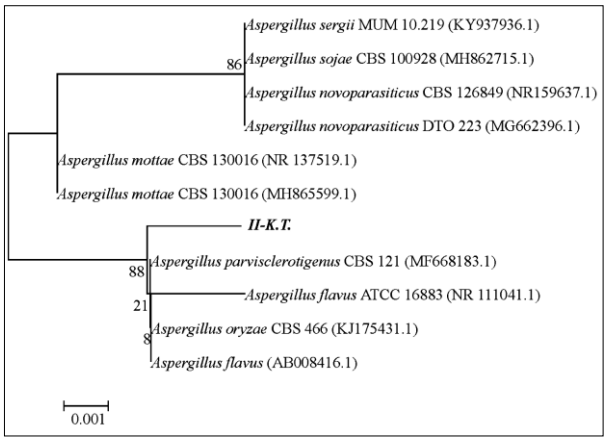

Fig 5 Report ID: C_SEP_19_009 Strain Details: Strain 1: II-K.T. Phylogenetic Tree

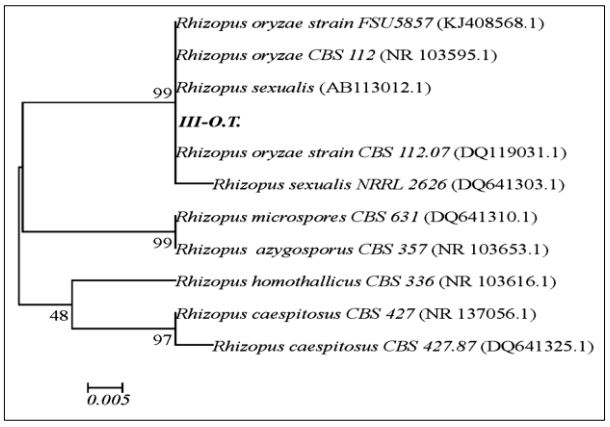

Fig 6 Report ID: C_SEP_19_010 Strain Details: Strain 1: III-O.T. Phylogenetic Tree

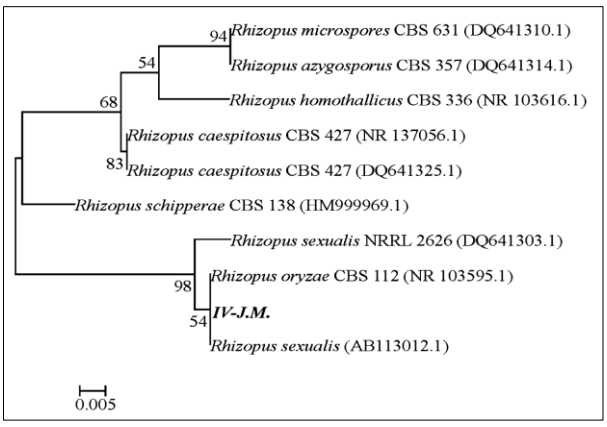

Fig 7 Report ID: C_SEP_19_011 Strain Details: Strain 1: IV-J.M. Phylogenetic Tree

Molecular identification of the isolated fungi

Eleven fungal isolates were identify on the basis of their molecular characteristics. Amplification of 18s rRNA with ITS primers has been successfully demonstrated and the 18s rRNA gene has been selected as a target for PCR amplification as a comprehensive molecular analysis of sequence data to reconstruct the evolutionary history of biocommunities is used to. The phylogenetic analysis was obtained by the neighbor-joining method based on single gene sequence for a total of ~ 500 bp length of the ITS region of your samples with its closest type strains in the database In the phylogenetic tree, three, strain of the sequenced 18S rRNA gene was identified as the 18SrRNA sequence analysis revealed that the isolate is close relative of *Aspergillus costaricaensis* isolates (gene bank accession no. MW001760) with different similarities (98.60%,98.16%,and 98.64%) (Table 1) next 2 fungal strain showed high level of 18S rRNA close neighbor of *Aspergillus costaricaensis* and no.

similarities(99.07% and 99.2%) of both species. Another strain similar (99.80%) with *Aspergillus aflatoxiformans* isolate *Aspergillus luchuensis* isolates (gene bank accession MW001760, MW001762) with different DTO 228-G2 (Accession no MG662344.1) next three strain close relative to *Rhizopus sexualis* var. *americanus* (Accession no MW001765) with different similarities (99.07%and 99.78%). Next strain similar (99.19%) with *Rhizopus oryzae* CBS112.07 (Accession no MW001767).

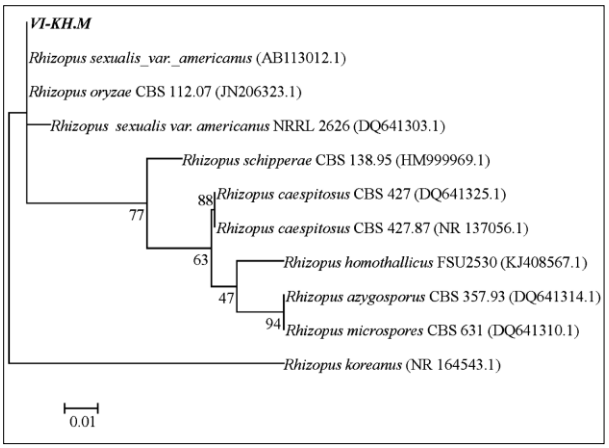

Fig 8 Report ID: C_SEP_19_013 Strain Details: Strain 1: VI-KH.M Phylogenetic Tree

Abdel Ghany *et al.* [16] identified *Aspergillus niger, A. fumigatus, A. sulphureus* and *A. flavus*. On the fungal swab cultures from False-door contained the same fungal species of *aspergilli*, with the other genus together with *Alternaria alternata, Alternaria sp*. and *Cladosporium herbarum*.

CONCLUSION

During screening for search of biocommunities, total six species of Microorganisms were isolated from different sandstone monuments. In these monuments *Aspergillus constaricaensis* shows maximum frequency, density as well as Abundance followed by *Rhizopus sexualis*. Some of the fungal species are confined to particular area i.e. *Rhizopus oryzae*, *Aspergillus luchuensis*, *Aspergillus aflatoxiformans*, *Rhizopus americanus*. These confinements of microorganism depend on environmental conditions of the area, which fluctuates from geographical area to area. In the present study Aspergillus species are the most common species found in the sites.

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