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ORIGINAL ARTICLE

Molecular Analysis of *Rhizopus* Fungal Species on Deteriorating Historical Sandstone Monuments

Swati Shakya*, Neelam Tripathi, Seema Bhadauria
Department of Biotechnology, Sri Satya Sai University of Technology and Medical Science Sehore (M.P.)
Email id- shakyaswati@yahoo.com

ABSTRACT
Sandstone monuments may support the broad categories of microorganism that are active in process of biodeterioration . Sandstone monuments have the compound of consortia of Bryophyte and fungi . Fungi is considered the most effective group of microorganism causing biodeterioration of sandstone monuments . Fungal units grows in the outdoor conditions in presence of low amount of nutrition and water it can start fungal colonization and the biodeterioration processes . Eleven fungal species were isolated having dominance over sandstone structures of the different monuments . During the investigation it was observe that Aspergillus species are dominant than Rhizopus species . Micro fungi cause discoloration and mechanical exfoliation of different monuments that was analyzed production of dark pigments , Hyphae penetration and organic Acid during the identification using molecular technique.
Keywords*: Sandstone, Microorganism, Fungi, Biodeterioration, Aspergillus, Rhizopus*

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INTRODUCTION

Fungi are known as one of the most harmful group of organism associated to biodeterioration of sandstone surfaces. The ability of fungi to colonize the substrate is greatly enhanced by different factor among which the most important are the environmental factors water availability and nutrients, special orientation and bio receptivity of artifact surface. The study of biocommunities in monuments is usually accomplished by using standard culture methods. However, it is believed that only small percent ages of extant microorganism have been discovered, and that cultured methods are in adequate for studying biocommunities composition [1]. Biocommunities in outdoor stone monuments represents a complex ecosystem including bacteria, algae, fungi, lichens. Biocommunities colonize sand stonework whenever the conditions of temperature, nutrition, moisture, and light are favorable. When microorganisms colonization is observable, the conservator should substantiate at which expanse it deteriorate the materials and know the non-biogenic agents that take part in the degradation[2]. Many causes have same effects, act in interact, or synergy in quantitatively fluctuting relations. Thus, the significant of biological impact to the entire deterioration process should be estimate very carefully. Observing microorganisms on cultural heritage objects does not automatically assume that they actually change the physical properties or chemical composition of the sandstone [3]. The present study to investigated the Isolation and Identification of Fungi by using RFLP and RAPD.

MATERIAL AND METHODS

The present paper deals with various methods used in analysis of biodeterioration of sandstone monument. The methodologies involve the identification of algae, fungi, bacteria and angiosperm.

Sampling

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 Browm 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 were 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 tungustun, 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 sequences are used to form contigs, which are then matched in well-curated databases for assigning closest neighbor as the tentative identification of your submitted organism.

RESULTS AND DISCUSSION

Investigation of different monuments resulted to analyze the effect on stone due to many Physical, chemical and biological factors. The alterations shown in the form of black patina, rusting of stone exfoliation. The present paper stated that microbial deterioration of archeological sandstone sample which is taken from eleven localities. The result revealed the isolation and identification of fungal strain that relate to *Aspergillus*. These similar results were showed by researcher [5-6]. The presence of *Aspergillus sp.* that include *Aspergillus costaricaensis* and *Aspergillus luchuensis.*

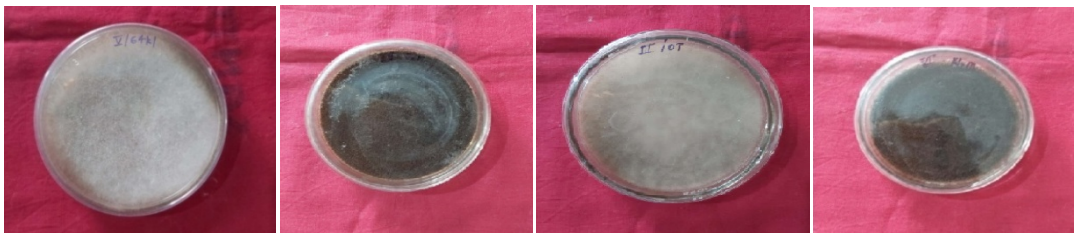

Fig.1 Isolated fungus

Identification

Samples 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*.

Molecular Identification Of The Isolated Fungi Eleven fungal isolates were identify on the basis of their molecular characteristics. The amplification of 18s rRNA with ITS primers has been successfully performed and 18s rRNA genes chosen as a target for PCR amplifications because the sequence data is widely used in the molecular analysis to reconstruct the evolutionary history of organism. The phylogenetic tree 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. MH862988.1) 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 MH862988.1, NR_135449.1) with different DTO 228-G2 (Accession no MG662344.1).next three strain close relative to *Rhizopus sexualis var. americanus* (Accession no AB113012.1) with different similarities (99.07%and 99.78%). Next strain similar (99.19%) with *Rhizopus oryzae* CBS112.07 (Accession no DQ119031.1).

Table 2 Identification of Fungus

PRN	Strain No.	Closest Neighbour	Accession No.	% Similarity
C_SEP_19_010	III-O.T.	***Rhizopus sexualis var. americanus***	**AB113012.1**	**99.07**
C_SEP_19_011	IV-J.M.	***Rhizopus sexualis var. americanus***	**AB113012.1**	**99.78**
C_SEP_19_012	V-61K	***Rhizopus oryzae CBS 112.07***	**DQ119031.1**	**99.19**
C_SEP_19_013	VI-KH.M	***Rhizopus sexualis var. americanus***	**AB113012.1**	**99.78**

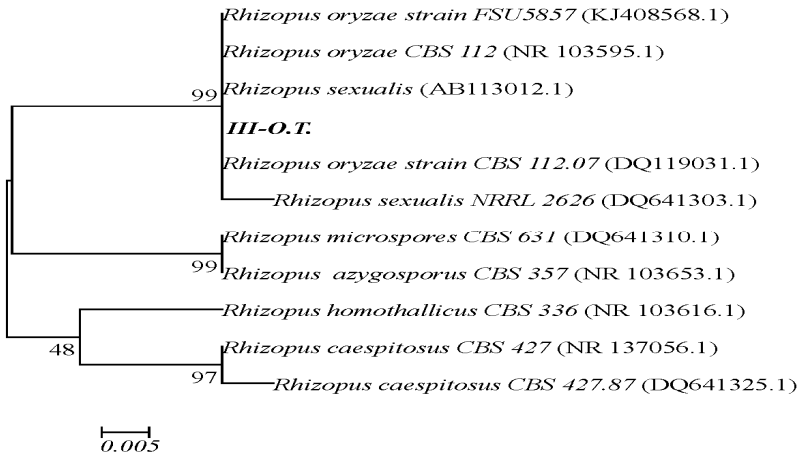

Fig.2 ***Phylogenetic Tree: Report ID:*** **C_SEP_19_010** *Strain Details:* ***Strain 1:*** **III-O.T.**

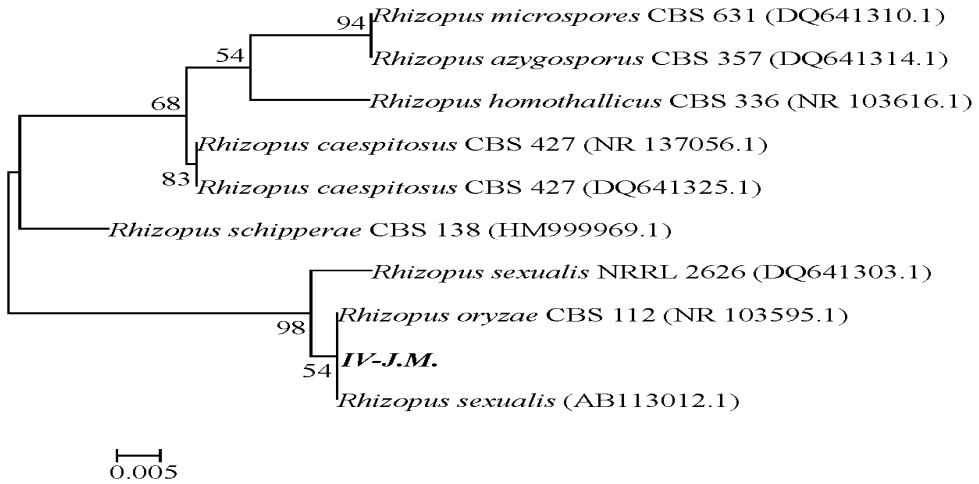

Fig. 3 ***Phylogenetic Tree: Report ID:*** **C_SEP_19_011** *Strain Details:* ***Strain 1:*** **IV-J.M.**

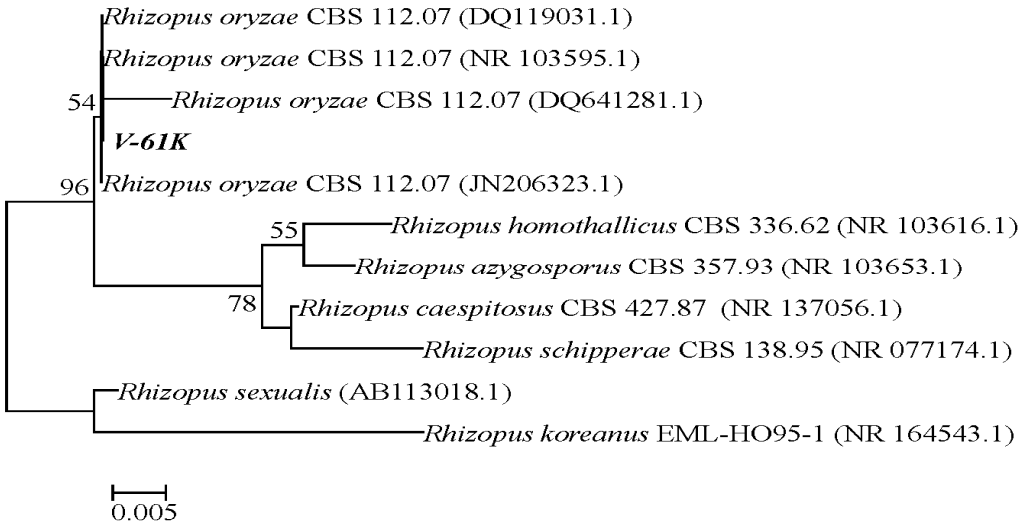

Fig.4 *Phylogenetic Tree:Report ID:* C_SEP_19_012 *Strain Details:* ***Strain 1: V-61K***

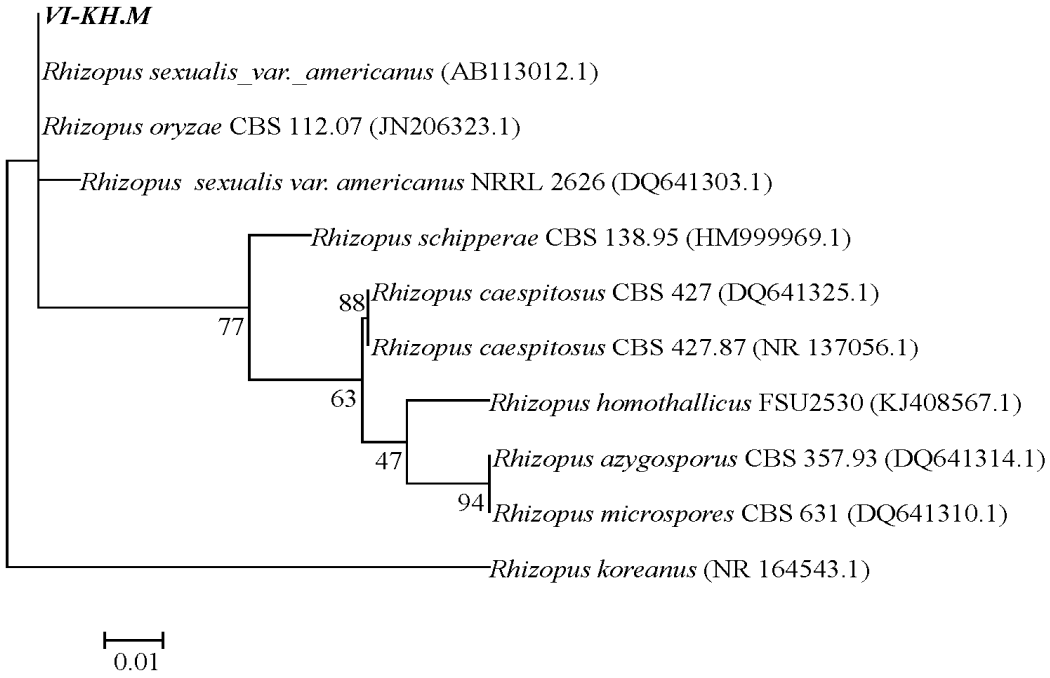

Fig. 5 *Phylogenetic Tree: Report ID:* C_SEP_19_013 *Strain Details:* ***Strain 1:*** **VI-KH.M**

Fig 2-5 Phylogenetic tree showing the relationship of closely relative species constructed using the neighbor-joining method and based on 18s rRNA genes sequences. Isolate is closely related to *Aspergillus*

Biological infections and the intensity of biodeterioration processes are strongly affected by water availability. This is determined by material-specific parameters, like porosity and permeability, environmental conditions of the site and exposure of the object. The establishment of fungi on sandstone is possible even without the pioneering participation of phototrophic organisms. Fungi are especially concentrated in stone crusts. These organisms are capable to get into the rock material by hyphal growth and biocorrosive activity, due to elimination of organic acids or by oxidation of mineral forming cations, preferably iron and manganese. The isolate protoplast from Rhizopus strain and carry out protoplast fusion to enhance numeric acid production from glycerol in fusant progenies [7]. Crude glycerol is the main biodiesel waste stream [8]. predict production of approximately 18.5 million of this by product by

year 2016. Its biotechnological utilization has been substantially developed during the last few years and various microbial metabolites. e.g. industrially important assets (citric, numeric, malic, oxalic and succinic), 1,3-propanediol,erythritol,alchohols, fungal protein and enzymes (lipase, phytase) have been produced [8-9]. Some rhypus strain can produced fumeric acid from glycerol but with the poor yield. The variation in structure of fungal organism depends upon, degree of competition between the prevailing environmental conditions and the fungal organisms [10]. The relative frequency and frequency are directly or indirectly correlated with climatic conditions and meteorological data. *Aspergillus niger* released certain metal ions from the rock Aspergillus are the most common species found in the sites and observed that *Aspergillus niger, Aspergillus flavus, Curvularia lunata* and *Rhizoctonia solan*i were found most dominant [11].

It can be concluded that surface of sand stone in the different monument are proper substrates for microbial colonization. In this research deal with isolation and identification of fungi characterization and detection of biodeteriogens are required before any conservation and restoration treatment further more investigation should be carried out to find the best method to control and remove of fungal growth on the surface of sand stone monument.

CONCLUSION

Fungal species were isolated from different sandstone monuments. The isolated species first identified morphologically and then tested. *Aspergillus* and *Rhizopus* species were identified genetically by sequencing-550 base pair.

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REFERENCES

1. Laiz, L., M. Gonzalez Delvalle, B. Hermosin, A. Ortiz Martinez and C. Saiz Jimenez (2003) Isolation of Cave Bacteria and Substrate Utilization at Different tempreture. Geomicrobiology Journol 20(5):479-489
2. Warscheid, T. and Braams, J. (2000) Biodeterioration of Stone: A Review. International Biodeterioration & Biodegradation, 46, 343-368
3. Pinna, P. (2014). Biofilm and lichens on stone monuments: do they damage or protect?. Frontiers in Microbiology, 5(133):133
4. Kumar, A.V. (2001). Conservation of Building stones Pub. (INTACH) Indian council of conservation institute Lucknow 229.
5. Mohammadi P. and N. Maghboli-Balasjin (2014). Isolation and molecular identification of deteriorating fungi from Cyrus the Great tomb stones. Iranian Journal of Microbiology, 6, No 5, 361-370.
6. Seth, R. K., Shah Alam and D. N. Shukla (2016). Isolation and identification of Soil Fungi from Wheat Cultivated Area of Uttar Pradesh. Journal of Plant Pathology & Microbiology, 7:11
7. Wiater- Kordowska, Magdalena Polak-Berecka, Adam Wasko, Zdzislaw Targonski (2012) protoplast fusion of *Rhizopus oryzae &Rhizopus microspores* for enhanced fumeric acid production from glycerol. Journal of biotechnology computational biology and bio nano technology,93(4) :425-430.
8. Yang F., Hanna M.A., Sun R. (2012) Value-added uses for crude glycerol – a by product of biodiesel production. Biotechnol. Biofuels 5: 13.
9. Nicol R.W., Marchand K., Lubitz W.D. (2012) Bioconversion of crude glycerol by fungi. Appl. Microbiol. Biotechnol.93: 1865-1875.
10. Chandel, D.S., (1990). Studies of Phylloplane interaction of fungi from Soybean and Pigeon pea. Ph.D. Thesis. Pt Ravishankar University, Raipur.
11. Gupta S.P., K.S. Rana, D. N. Sharma, G. K. Chandrol (2013). Diversity and index of similarity of microorganisms on sandstone with special reference to historical monuments of Chhattisgarh, India. International journal of current microbiology and applied science, 2(12): 51-57.