ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 2, pp.2550-2553, February, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

PRESERVATION OF PRE-HISTORIC MONUMENTS AND BUILDINGS FROM THE EFFECT OF DESERT VARNISH

Joshi.A[1*] and Harwani D[1]

[1*]National Institute of Animal Biotechnology, Hyderabad, Andhra Pradesh, India
[1]Maharaja Ganga Singh University, Bikaner, Rajasthan, India

ARTICLE INFO

Article History:

Received 5th, January, 2015
Received in revised form 12th, January, 2015
Accepted 6th, February, 2015
Published online 28th, February, 2015

Key words:

Desert varnish, Bacteria, Bergey's manual, Preservation of monuments.

ABSTRACT

Desert varnish is the thin red to black coating found on exposed rock surfaces in arid regions. Rajasthan, which is located on the western side of India and a part of the Great Indian Desert, has artistic and cultural traditions which reflect the ancient Indian way of life. There is a rich and varied folk culture and ancient monuments which are often depicted symbolic of the state. It makes Rajasthan very important for tourism perspective. Latterly, almost all prehistoric forts and monuments of Rajasthan are affected from dark coatings of desert varnish and losing their elegance and charm, which makes it very imperative to find suitable measures to bring back the attraction of these monuments.

INTRODUCTION

Rajasthan (the land of kings) is famous in the world for its rich culture, deserts, historic monuments, Forts and buildings. This made it one of the most popular tourist destinations in India. According to Tourism Annual Report 2012-2013, Department of Tourism, Rajasthan, more than 30 million domestic and foreign visitors visited Rajasthan in 2012. The gracefulness and attraction of ancient forts and monuments is necessary to be maintained by archaeological and tourism point of view.

In recent times, ancient forts and monuments of Rajasthan are facing a major problem called desert varnish. It is a thin red to black coating found on exposed rock surfaces in arid regions. It is composed of clay minerals, oxides and hydroxides of manganese and iron, cemented to rock surfaces. Dorn and Oberlander, (1981) firstly observed that the desert varnish is a product of microbial activity and is composed of micro-organisms. Desert varnish is mostly made up of clay minerals and Iron and Manganese oxides. These two oxides give the desert varnish it's red or black colours, respectively. Endospore forming microbes, specifically Gram positive bacteria, are responsible for its Manganese oxidation. These include Micrococcus, Planococcus, Arthrobacter, Deodermatophilus, and Bacillus. Dying bacteria secrete amino acids, also contributing to the varnish coating and biominerals. (Rakovan 2006). It occurs on nearly all rock types, including quartzite, which have little or no iron, manganese, and clay minerals (Thiagarajan and Lee, 2004), Interestingly, in Rajasthan mostly all forts and palaces are made of sandstone which is composed of quartz.

Desert varnish is directly involved in the weathering of ancient forts and buildings (Figure1) and because of this, the attraction and gracefulness of ancient monuments is decreasing year to year. So to overcome this problem, it is very important to detect the microbial communities involved in desert varnish formation and to discover effective measures to prevent desert varnish from outer surfaces of ancient forts.

Figure1 Desert Varnish growing on an ancient building located at Bikaner city of Rajasthan

MATERIALS AND METHODS

To collect scrapings of Desert Varnish, different locations were selected. Samples were collected from different

Corresponding author:* **Joshi. A
Maharaja Ganga Singh University, Bikaner, Rajasthan, India

monuments located at three different regions in Bikaner city (Rajasthan, India). Sampling was done aseptically in day time when temperature was ranging between 34-42 ^{0}C, for understanding the microbial diversity of desert varnish, samples were serially diluted and inoculated separately on the Nutrient agar and Luria Bertani plates. Bacterial colonies in pure form were collected using streak plate method. From the mixed culture a single colony was picked and sub cultured on a fresh medium plate, by quadrate streak plate method to obtain pure bacterial colonies. Based on phenotypic colony characters on plate, total 23 different bacterial colonies were isolated in pure forms, which were further characterized and identified using various methods.

The characterization and identification of bacteria was done with the help of Bergey's Manual of Systematic Bacteriology, which is the main resource for determining the identity of bacterial genera, utilizing every characterizing aspect and distinguishing bacterial species based on phenotypic differences between isolates. Primary characterization of

Table 1 Number of bacterial genus isolated from desert varnish

Bacterial Genus	No. of strains identified
Bacillus	7
Corynebacterium	5
Micrococcus	3
Streptococcus	5
Aeromonas	1
Erysipelothrix	2

Table 2 Optical Densities of all isolated bacterial strains when culturing with media containing calcium carbonate and wall paint.

Bacterial Genus	O.D of control (nutrient broth)		O.D of media containing calcium chloride		O.D of media containing wall paint	
	Day 1 (24 Hr)	Day 2 (48 Hr)	Day 1 (24 Hr)	Day 2 (48 Hr)	Day 1 (24 Hr)	Day 2 (48 Hr)
Bacillus	1.610	2.101	.123	.160	.155	.225
Corynebacterium	1.768	3.293	.236	.352	.290	.410
Micrococcus	1.327	2.223	.539	.756	.530	.816
Streptococcus	1.548	2.543	.223	.368	.350	.451
Aeromonas	1.572	2.983	.501	.692	.550	.710
Erysipelothrix	1.392	2.137	.195	.249	.220	.392

Supplementary Table 1 Results for test of phenotypic and microscopic characters of all isolated bacterial strains

	Phenotypic Characteristics						Cell Morphology	Gram reaction (+/-)	O2 Use +,+/-,_	Endospore (Y/N)	Motile (Y/N)
	Size (mm)	Shape	Elevation	Color	Edge	Surface					
A1	.2 mm	Round	Raised	Yellow	Irregular	Shiney	Coccus	+	+/-	N	N
A2	.4 mm	Irregular Round	Convex	Cream	Entire	Shiney	Coccus	+	+	N	N
A3	.15mm	Round	Flat	Cream	Irregular	Shiney	Rod	+	+	Y	N
A4	.3 mm	Round	Raised	Yellow	Entire	Shiney	Coccus	+	+/-	N	N
A5	.20mm	Round	Raised	Yellow	Entire	Shiney	Rods	+	+/-	Y	N
A6	.20mm	Round	Flat	Cream	Entire	Smooth	Rods	+	+	Y	N
A7	.10mm	Irregular	Flat	Yellow	Irregular	Smooth	Strepto Coccus	+	+/-	N	N
A8	.8mm	Irregular	Raised	Orange	Irregular	Smooth	Strepto Coccus	-	+/-	N	N
A9	.12mm	Irregular	Raised	Yellow	Entire	Smooth	Strepto Coccus	+	+/-	N	N
A10	.30mm	Round	Raised	Cream	Entire	Smooth	Filamentous	+	+	N	N
A11	.25mm	Round	Raised	Peach	Entire	Smooth	Rods	+	+	Y	N
A12	.22mm	Irregular	Flat	Cream	Irregular	Smooth	Long Rods	+	+/-	N	N
A13	.35mm	Irregular	Flat	Cream	Irregular	Smooth	Filamentous	+	+	N	N
A14	.40mm	Irregular	Flat	Cream	Irregular	Smooth	Rods	+	+	Y	N
A15	.18mm	Round	Flat	Cream	Entire	Smooth	Rods	+	+	Y	N
A16	.33mm	Round	Raised	Cream	Entire	Smooth	Rods	+	+/-	N	N
A17	.15mm	Round	Raised	Cream	Irregular	Shiney	Long Rods	+	+/-	N	N
A18	.22mm	Round	Raised	Yellow	Entire	Shiney	Rods	-	+/-	N	N
A19	.10mm	Round	Flat	Cream	Entire	Smooth	Strepto Coccus	+	+/-	N	N
A20	.14mm	Round	Raised	Cream	Entire	Smooth	Rods	+	+/-	N	N
A21	.20mm	Irregular	Raised	Cream	Entire	Smooth	Rods	+	+/-	Y	N
A22	.19mm	Round	Raised	Cream	Entire	Smooth	Strepto Coccus	+	+/-	N	N
A23	.32mm	Irregular	Raised	Cream	Entire	Smooth	Rods	+	+/-	N	N

*Serial number A1 – A23 indicates the number of bacterial colonies isolated from 3 samples of Desert Varnish on nutrient agar and Luria bertani plates separately.

isolated bacteria was done using visual characters (colony shape, color and elevation), microscopic characterization (Gram and Endospore staining) and some other tests like motility test, oxygen tolerance test, catalase test and the secondary identification of the isolates was carried out on the basis of biochemical tests (IMViC tests) and carbohydrate utilization test. In order to see and search effective curing against desert varnish, wall paint and calcium chloride (easily available and cost-effective) were selected to check their effect on the growth of bacteria isolated from the desert varnish. For this purpose bacterial strains were inoculated in nutrient broth medium containing 1% of wall paint and calcium chloride. This was incubated for 48 hours at 37^{o}C. The optical density was observed on first and second day (at 24 and 48 hours) at 560nm of wavelength using spectrophotometer.

RESULT AND DISCUSSION

The present study was conducted to access the microbial diversity of desert varnish of old monuments and buildings of Bikaner region. First of all 23 bacterial were isolated in pure cultures and were further subjected to identification and characterization process as described in the Bergey's Manual of Systematic Bacteriology. According to this, bacteria were characterized on the basis of phenotypic characters, cell morphology and biochemical tests.

To see results for phenotypic characters and biochemical tests please see supplementary table 1 and 2. The following table reveals the diversity of bacterial genera found in desert varnish. Desert varnish is capable of preserving microbial fossilization, which includes bacterial casts, and it is also responsible for weathering of archaeologically important forts and buildings.

In the present research bacterial genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Streptococcus*, *Aeromonas* and *Erysipleothrix* were found to be present in desert varnish. The outcome of this project also directs us that there is an immediate need to preserve and secure our ancient monuments and forts from weathering and decay due to desert varnish. So to overcome this problem, Bacterial genera obtained from desert varnish scrapings were inoculated with density at 24 and 48 hours by keeping control (without paint wall paint and calcium chloride and observed their optical and calcium chloride) as reference.

As shown in the table 2 when desert varnish bacteria were cultured with 1 % calcium chloride, on day first *Micrococcus* Showed maximum growth followed by *Aeromonas* and *Corynebacterium.*

The growth of all bacterial strains was assessed to be high when grown without any chemical agent, but when calcium chloride was added in medium, the bacterial growth showed significant decline, which was assessed by taking optical density at day 1 and day2. In table 2, it is clearly shown that when desert varnish bacteria were cultured in nutrient broth without wall paint and calcium chloride, the growth was observed to be pronounced (as shown as O.D in table 2).

But the use of either wall paint or calcium chloride in the medium negatively affect bacterial growth and could act as a effective antimicrobial to cure old monuments and forts from bacteria dwelling on them. When the same bacteria cultured in nutrient agar with 2% calcium chloride and wall paint separately, the optical densities were observed negligible.

Supplementary table 2 Results of biochemical test carried out with all 23 bacterial strains isolated from Desert Varnish samples.

S. No.	Catalase	Indole	Methyl Red	Voges Proskauer	Mannitol	Dextrose
A1	Positive	Negative	Negative	Negative	High	Average
A2	Positive	Negative	Negative	Negative	average	Average
A3	Positive	Negative	Negative	Negative	Spare	High
A4	Positive	Negative	Negative	Negative	High	Average
A5	Positive	Negative	Negative	Negative	Spare	High
A6	Positive	Negative	Negative	Negative	Spare	High
A7	Negative	Negative	Negative	Negative	Average	Average
A8	Negative	Negative	Negative	Negative	Average	Average
A9	Negative	Negative	Negative	Negative	Average	Average
A10	Positive	Negative	Negative	Negative	Spare	High
A11	Positive	Negative	Negative	Negative	Spare	High
A12	Positive	Negative	Negative	Negative	Spare	High
A13	Positive	Negative	Negative	Negative	Spare	High
A14	Positive	Negative	Negative	Negative	Spare	High
A15	Positive	Negative	Negative	Negative	Spare	High
A16	Positive	Negative	Negative	Negative	Spare	High
A17	Positive	Negative	Negative	Negative	Spare	High
A18	Positive	Negative	Negative	Negative	Spare	High
A19	Negative	Negative	Negative	Negative	Average	Average
A20	Positive	Negative	Negative	Negative	Spare	High
A21	Positive	Negative	Negative	Negative	Spare	High
A22	Negative	Negative	Negative	Negative	Average	Average
A23	Positive	Negative	Negative	Negative	Spare	High

Recommendations and Outcomes

1. The gracefulness and attraction of ancient monuments is necessary to maintain by archaeological and historical point of view.
2. Use of wall paint works as antimicrobial agent and negatively affects Desert Varnish.
3. Use of 1% Calcium Chloride solution is also recommended to cure Desert Varnish from ancient buildings and monuments.

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How to cite this article:

Joshi. A and Harwani. D: Preservation of pre-historic monuments and buildings from the effect of desert varnish. *International Journal of Recent Scientific Research Vol. 6, Issue, 2, pp.2550-2553, February, 2015*

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