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Diversity of cyanobacteria on stone monuments and building facades of India and their phylogenetic analysis

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ABSTRACT

Many archaeologically important stone temples, caves, mortar monuments with artistic expression as well as building facades of India are now disfigured due to colonization of cyanobacterial biofilms leading to weathering of the substratum. They are composed of species principally belonging to the genera *Hassallia, Tolypothrix*, *Scytonema, Lyngbya* and *Calothrix*, which appeared soon after wetting of the biofilms. Several other species of genera *Aulosira*, *Nostoc*, *Camptylonema, Dichothrix*, *Chlorogloeopsis* and *Westiellopsis* occurred as associated organisms as they appeared upon prolonged culture of the biofilms. Molecular phylogenetic analysis based on 16S rRNA partial gene sequencing of all these 24 cyanobacteria species under 11 genera isolated from the surfaces of monuments and building facades of India along with those of other species isolated from stone surfaces in subaerial habitats and hypogeal environments in different regions of the globe showed that those species from tropical regions were clustered together, quite different from cyanobacteria of the genera *Chroococcidiopsis, Leptolyngbya*, *Phormidium*, *Nostoc*, *Rexia*, *Symphyonemopsis*, *Scytonema*, *Tolypothrix* and *Calothrix* occurring in the temperate regions.

1. Introduction

The exposed surfaces of stone temples, monuments as well as building facades in most regions of India look blackish colour due to formation of phototrophic biofilms. The principal species there-in are certain species of cyanobacteria (Roy et al., 1997; Tripathy et al., 1997). They could survive in the biofilms even during summer months when the temperature on these structures exceeds 60 °C coupled with high light intensity and extreme dryness (Tripathy et al., 1999; Pattanaik and Adhikary, 2002; Samad and Adhikary, 2008; Keshari and Adhikary, 2013). These organisms sprout receiving moisture on the onset of monsoon and grow utilizing the minerals leading to deterioration of monuments of cultural value (Gaylarde and Morton, 1999; Gaylarde and Gaylarde, 2000, 2001; Crispim et al., 2003). Certain species of cyanobacteria possess well developed sheath layers around their trichome containing UV-sunscreen pigments like scytonemin and MAAs (Garcia-Pichel and Castenholz, 1991, 1993; Adhikary and Sahu, 1998; Mandal and Rath, 2012) and secrete copious extracellular polymeric substances (EPS) (Bertocchi et al., 1990; Gloaguen et al., 1995; Adhikary, 1998; Rossi et al., 2012), all of which contribute to their protection against desiccation and intense solar radiation. Due to their phototrophic nature and many being nitrogen fixers, cyanobacteria colonize easily on exposed surfaces that in due course lead to formation of patinas and incrustations causing aesthetic damage.

Cyanobacteria being important from evolutionary and ecological point of view, the mode of their diversity analysis and taxonomy are changing with recent information and techniques. Very little information is available on identification and phylogeny of cyanobacteria species from Indian environments following molecular approach (Sahu and Adhikary, 2012; Keshari and Adhikary, 2013). Organisms occurring in desiccated state on sub-aerial surfaces are difficult to identify following available monographs as seldom the morphological features of the species can be seen even after prolong wetting of the natural material. Further, when adapted to extreme environments they undergo structural adaptation, thus identification and determining their evolutionary relationship is difficult. Hence 16S rRNA gene sequences of cyanobacteria species isolated from stone temples and monuments as well as on building facades from several locations of India were obtained and their molecular phylogeny was determined comparing the gene sequences of species from identical surfaces of other regions of the globe.

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Table 1
List of major and associated species of cyanobacteria isolated from the exterior of different stone monuments, caves and building facades of India.

Cyanobacteria	Place of collection	Substratum	Major species	Associated species
Hassallia byssoidea Hassall (Fig. 2, a)	Sun temple, Konark, Odisha	Stone surface	+	
Dichothrix baueriana Bornet et Flahault (Fig. 2, b)	Sun temple, Konark, Odisha	Stone surface		+
Tolypothrix scytonemoides (Gardner) Geitler (Fig. 2, c)	Kedar-Gouri temple, Bhubaneswar, Odisha	Stone surface	+	
Nostoc carneum Agardh ex Bornet et Flahault (Fig. 2, d)	Kedar-Gouri temple, Bhubaneswar, Odisha	Stone surface		+
Tolypothrix distorta var. *penicillata* Kützing ex Bornet et Flahault (Fig. 2, e)	Jogin temple, Hirapur, Bhubaneswar, Odisha	Stone surface	+	
Nostoc commune Voucher ex Bornet et Flahault (Fig. 2, f)	Lingaraj temple, Bhubaneswar, Odisha	Stone surface		+
Nostoc linckia (Roth) Bornet ex Bornet et Flahault (Fig. 2, g)	Rameswar temple, Bhubaneswar, Odisha	Stone surface		+
Lyngbya aerugineo-coerulea (Kützing) Gomont (Fig. 2, h)	Brahmeswar temple, Bhubaneswar, Odisha	Stone surface	+	
Nostoc punctiforme (Kützing) Hariot (Fig. 2, i)	Nrusinghanath temple, Paikamal, Odisha	Stone surface		+
Chlorogloeopsis fritschii Mitra et Pandey (Fig. 2, j)	Vrihadeswar temple, Thanjavur, Tamil Nadu	Stone surface		+
Lyngbya kuetzingiana Kirchner (Fig. 2, k)	Tiger cave, Khandagiri, Bhubaneswar, Odisha	Stone surface	+	
Tolypothrix bouteillei (Brebissom et Desm.) Forti (Fig. 2, l)	Ajanta caves, Maharashtra	Stone surface	+	
Calothrix gardneri De Toni (Fig. 2, m)	Ajanta caves, Maharashtra	Stone surface		+
Scytonema coactile Montagne ex Bornet et Flahault (Fig. 2, n)	Kutumsar cave, Chhattisgarh	Stone surface	+	
Scytonema millei Bornet ex Bornet et Flahault (Fig. 2, o)	Buddha statue, Santiniketan, West Bengal	Stone and mortar	+	
Scytonema chiastum Geitler (Fig. 2, p)	Buddha statue, Santiniketan, West Bengal	Stone and mortar		+
Westiellopsis prolifica Janet (Fig. 2, q)	Buddha and Gandhi statue, Santiniketan, West Bengal	Stone and mortar		+
Tolypothrix campylonemoides Ghose (Fig. 2, r)	Gandhi statue, Santiniketan, West Bengal	Stone and mortar	+	
Aulosira pseudoramosa Bharadwaja (Fig. 2, s)	Gandhi statue, Santiniketan, West Bengal	Stone and mortar		+
Scytonema sp. (Fig. 2, t)	Elephant sculpture, Santiniketan, West Bengal	Stone and mortar	+	
Calothrix marchica lemmermann (Fig. 2, u)	Rabindra Bhavana and Siksha Bhavana, Santiniketan, West Bengal	Building Facades	+	
Scytonema rivulare Borzi ex Bornet et Flahault (Fig. 2, v)	Patha Bhavana, P.M. hospital, Silpa Sadan and Santisree hostel, Santiniketan, West Bengal	Building Facades	+	
Camptylonema indicum Schmidle (Fig. 2, w)	Siksha Bhavana, Santiniketan, West Bengal	Building Facades		+
Tolypothrix rechingeri (Wille) Geitler (Fig. 2, x)	Siksha Bhavana, Santiniketan, West Bengal	Building Facades		+

2. Material and methods

2.1. Description of sampling sites

Eight stone temples, three caves, three mortar monuments and six building facades from different regions of India (Table 1, Fig. 1) were sampled for biofilms occurring on their exterior surfaces. Of these Sun temple of Konark; Kedar-Gouri, Jogin, Lingaraj, Rameswar and Brahmeswar temples of Bhubaneswar (19°80′ to 20°24′N, 85°87′ to 86°9′E) and Nrusinghanath temple of Paikamal (20°68′N, 82°46′E) were from Odisha and Vrihadeswar temple of Thanjavur (10°46′N, 79°7′E) from Tamil Nadu. These were built during 6th to 16th A.D., hence important from archaeological point of view. Besides the Tiger cave of Khandagiri (20°20′N, 85°44′E) from Odisha, Ajanta caves (20°33′N, 75°42′E) famous for artistic expressions and fresco-paintings from Maharashtra, Kutumsar cave (19°07′N, 82°03′E) from Chhattisgarh and the monuments made of mortar and the building facades of Santiniketan (23°68′N, 87°68′E), a cultural heritage site in West Bengal were also sampled.

2.2. Collection, observation, culturing and identification

Blackish-brown biofilms were collected from all the sampling locations soon after monsoon rain and stored in pre-sterilized sampling bottles. During the other seasons it was difficult to remove the biofilms from the stone surfaces, hence were collected following non-destructive method by employing adhesive tape strips (La Cono and Urzi, 2003). The biofilms were soaked in sterile distilled water and incubated under fluorescent light for up to 72 h and observed microscopically. Since the morphological features needed for identification were not distinct even after prolonged soaking (up to 7 days), a small amount of each sample was transferred to BG 11 medium with and without nitrogen (Rippka et al., 1979) and to agar plates (1.2% w/v agar in the same medium).

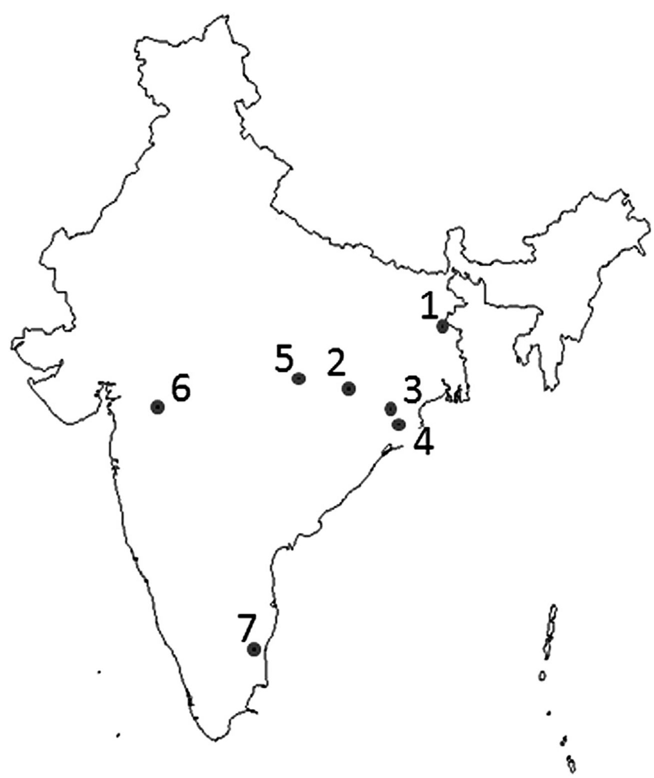

Fig. 1. Map of India showing sampling sites for cyanobacterial biofilms on exterior surfaces of temples and monuments in different locations of India. 1: Santiniketan (West Bengal), 2: Paikamal (Odisha), 3: Bhubaneswar (Odisha), 4: Konark (Odisha), 5: Kutumsar cave (Chhattisgarh), 6: Ajanta caves (Maharashtra) and 7: Thanjavur (Tamil Nadu).