Microbial deterioration of heritage monuments in Santiniketan, West Bengal, India

Microbial deterioration of monuments and facades of heritage buildings is a well-known and a widely recognized problem in India and the world over. Several stone monuments of archaeological importance, belonging to period from the 1st to 12th century AD in many regions of India have become obliterated, defaced and disfigured due to the colonization of blackish/brownish growth of biofilms on their exposed surfaces, causing aesthetic/structural disfigurement and damage[1,2]. The main species of these biofilms thriving on Indian monuments are cyanobacteria, which are stress-tolerant and can survive in the adverse condition of desiccation and extreme temperature of stones[3–5]. During the onset of monsoon, these blackish/brownish biofilms become greenish after absorbing moisture and utilize the minerals present in the stone substrata to grow. This is the main cause of deterioration of these important monuments[6–8]. Certain species of cyanobacteria which dominate these biofilms, develop survival strategies by secreting a thick sheath of extracellular polymeric substances (EPS) resulting into crust formation under adverse conditions and desiccation[9,10]. These cyanobacteria are also rich in UV-sunscreen pigments like scytonemin and mycosporine-like amino acids (MAAs)[11] which act as a protective mechanism against desiccation and intense solar radiation[12,13].

Earlier research on the diversity of cyanobacteria colonizing Indian stone monuments was based on traditional morphological studies[1,3–5]. However, the mode of their diversity analysis and taxonomy is changing with recent information and techniques, including different phenotypic features (e.g. biochemical and ultra-structural characteristics) and genotypic characterization[2,14–18]. The protection of culturally important monuments is of great concern to all mankind with the aim to contribute to the conservation of these universal heritage art treasures for future generations. The present study describes a novel cyanobacterial species, i.e. *Hassallia lithophila* from stone monuments of Santiniketan, West Bengal, India.

Blackish-brown biofilm was collected from a stone-made elephant sculpture (Figure 1 *a*) in Santiniketan (23°40.427′N, 87°40.581′E, 56 m amsl). Culture was grown in BG 11 medium[19] at 25° ± 1°C under continuous light with fluorescent tubes at an intensity of 7.5 W m^{-2}. Microscopic study was performed (Nikon microscope Ni–11 attached with Nikon Digital Camera DS–Ri1–U3 and Nikon Imaging Software NIS–D+EDF) and the type specimen was deposited at the Central National Herbarium (CAL), Howrah. Genomic DNA was extracted following a bacterial genomic DNA isolation protocol[2]. PCR amplification of 16S rRNA genes was carried out using CYA359F and CYA781R (equimolar mixture of CYA781R-a and CYA781R-b) primers specific for cyanobacteria[20]. The products were purified and sequenced (GCC Biotech, Kolkata). The sequence of *H. lithophila* was deposited in GenBank with accession no. JX523945 (initially mislabelled as *Scytonema* sp.). The phylogenetic tree was constructed using Mega-6.0 software[21].

Taxonomy – *Hassallia lithophila* N. Keshari, Sudipta K. Das & S.P. Adhikary sp. nov. (Figure 1).

Thallus rounded bushy mats, blue–green or black–green in colour; filaments heteropolar, cylindrical, fasciculated, 7–20 µm width; sheath slender, hyaline, non-lamellated, colourless in younger filaments, golden-yellow in mature ones (Figure 1 *b–d*); sheath parallel throughout the filament, but diffused towards the

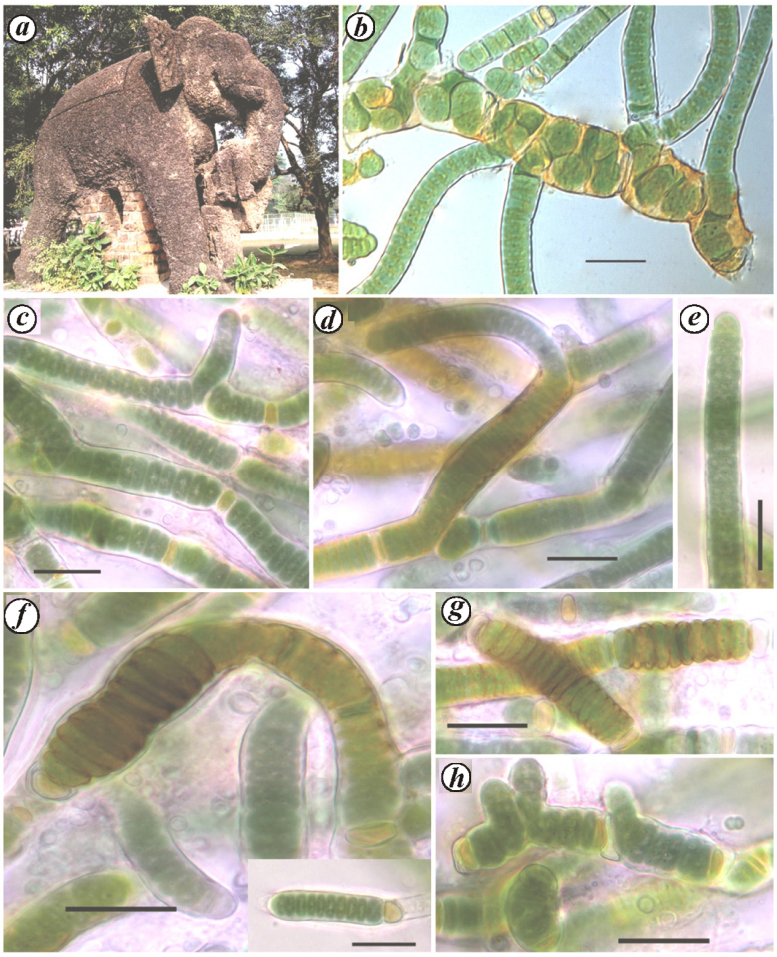

Figure 1. Habitat and photomicrographs of *Hassalia lithophila* sp. nov. ***a***, Elephant sculpture at Santiniketan. ***b–d***, Thallus, filament structure and branching. ***e***, Apical portion of the filament. ***f***, Reproduction through hormogonium. ***g***, Gelatinized filaments. ***h***, Tolypotrichoid pseudo-branches. (Scale bar: ***b–h*** = 20 µm.)

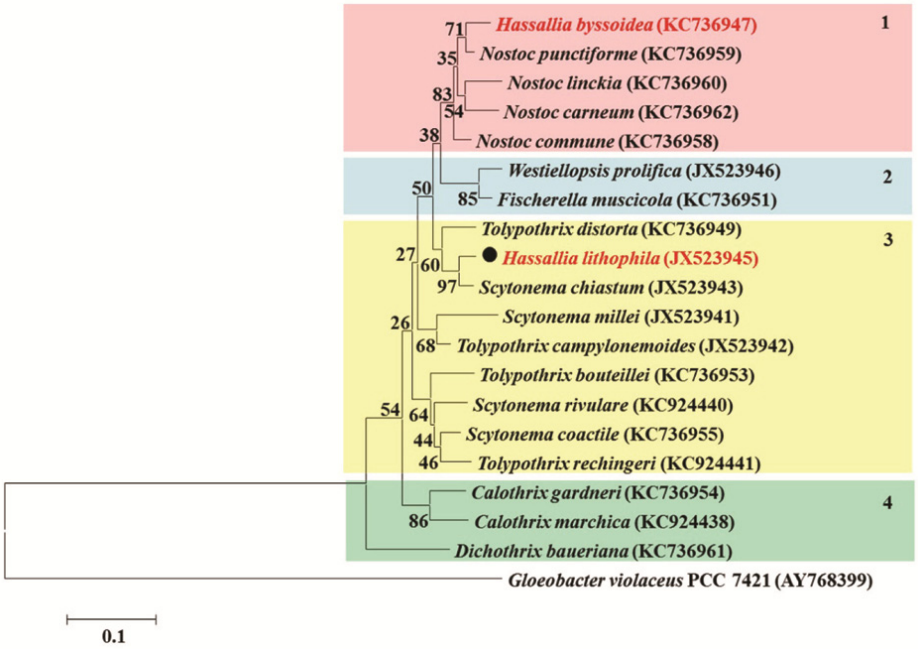

Figure 2. Phylogenetic relationship of *H. lithophila* sp. nov. with other taxa of similar habitat based on 16S rRNA partial gene sequences using the neighbour-joining method. Sequence accession numbers of these cyanobacteria retrieved from the GenBank are given within parenthesis.

apex, gelatized (Figure 1 *g*); pseudo-branches short, irregular, lateral, mature filaments mostly with double pseudo-branches and younger ones with single tolypotrichoid pseudo-branches (Figure 1 *c*, *d* and *h*); trichomes cylindrical, cross-walls clearly constricted, sometimes constriction not distinct in the apical portions (Figure 1 *e*); trichomes densely entangled in mature filaments giving a wavy appearance (Figure 1 *b*); cells broadly elliptical to barrel-shaped, bright blue–green in colour, shorter than wide, 4.6–17.2 µm in breadth, 2.5–8.3 µm in length; heterocytes both basal and rarely intercalary (Figure 1 *c* and *h*), solitary or in pairs, elliptical; reproduction is by hormogonia (Figure 1 *f*).

Occurred as a blackish crust on the stone monument (elephant) in Santiniketan.

Holotype – India: Santiniketan, West Bengal 23°40.427′N, 87°40.581′E (ca. 56 m), 19.07.2011, VB 3, *Nitin Keshari* (Holotype CAL!, Alg.054), cultures deposited at the algal culture collection in the Department of Biotechnology, Siksha Bhavana, Visva-Bharati, Santiniketan, with reference strain number VB511292.

Etymology – The epithet for the species was selected according to the habitat (stone surface) in which it occurred.

Phenotypical characters like heteropolar filaments, and tolypotrichoid false branches identified the present taxon as a member of the genus *Hassallia*, family Microchaetaceae. All the species of *Hassallia* have mostly overlapping characters, segregated from each other in sheath structure, particularly at the apices, trichome morphology and cytological dimensions. The present taxon is distinguished from other species, specifically the closely similar type species *Hassallia byssoidea* in densely entangled trichome in mature filaments and gelatinization of sheath. The phylogenetic tree was constructed based on 16S rRNA gene sequences of *H. lithophila* and other heterocystous cyanobacteria reported from the Indian terrestrial habitats using the neighbour-joining method (Figure 2). *Gloeobacter violaceus* PCC 7421 (AY768399) was used as the out-group. Dendrogram represented the clustering of similar types of genera close to each other, i.e. *Nostoc* in group-1, *Westiellopsis* and *Fischerella* in group-2, *Scytonema* and *Tolypothrix* in group-3 and *Calothrix* and *Dichothrix* in group-4. Moreover, *H. lithophila* clustered with *Scytonema* and *Tolypothrix* separated from other heterocystous genera like *Nostoc*, *Westiellopsis*, *Fischerella*, *Calothrix* and *Dichothrix*. *H. byssoidea* clustered with different species of *Nostoc* in group-1 away from *H. lithophila*. Phylogenetically, *H. lithophila* does not exhibit closeness to *H. byssoidea*, isolated earlier from the stone surface of Brahmeswar temple in Bhubaneswar (eastern India)[18], which was also supported by its distinct dissimilarity in morphology from *H. byssoidea*. Based on phylogenetic analysis and morphological evidence, we propose the present taxon as a new species.

1. Adhikary, S. P., *Indian J. Microbiol.*, 2000, **40**, 67–81.
2. Keshari, N. and Adhikary, S. P., *Biofouling*, 2013, **29**(5), 525–536.
3. Roy, A., Tripathy, P. and Adhikary, S. P., *Algol. Stud.*, 1997, **86**, 147–161.
4. Samad, L. K. and Adhikary, S. P., *Algae*, 2008, **23**(2), 91–114.
5. Tripathy, P., Roy, A., Anand, N. and Adhikary, S. P., *Feddes Repert.*, 1999, **110**(1–2), 133–144.
6. Scheerer, S., Ortega-Morales, O. and Gaylarde, C., *Adv. Appl. Microbiol.*, 2009, **66**, 97–139.
7. Dakal, T. C. and Cameotra, S. S., *Environ. Sci. Eur.*, 2012, **24**(1), 1.
8. McNamara, C. J. and Mitchell, R., *Front. Ecol. Environ.*, 2005, **3**(8), 445–451.
9. Pócs, T., *Acta Bot. Hung.*, 2009, **51**(1–2), 147–178.
10. Rossi, F., Micheletti, E., Bruno, L., Adhikary, S. P., Albertano, P. and DePhilippis, R., *Biofouling*, 2012, **28**(2), 215–224.
11. Adhikary, S. P. and Sahu, J. K., *J. Plant Physiol.*, 1998, **153**, 770–773.

12. Oren, A. and Gunde-Cimerman, N., *FEMS Microbiol. Lett.*, 2007, **269**(1), 1–10.
13. Sinha, R. P. and Häder, D. P., *Plant Sci.*, 2008, **174**(3), 278–289.
14. Keshari, N. and Adhikary, S. P., *Int. Biodeterior. Biodegrad.*, 2014, **90**, 45–51.
15. Keshari, N. and Adhikary, S. P., In *Cyanobacteria: An Economic Perspective* (eds Sharma, N. K., Rai, A. K. and Stal, L. J.), Wiley Blackwell, UK, 2013, pp. 73–90.
16. Keshari, N., Das, S. K. and Adhikary, S. P., *Eur. J. Phycol.*, 2015, **50**(4), 395–399.
17. Keshari, N., Das, S. K. and Adhikary, S. P., *Phytotaxa*, 2016, **283**(2), 181–187.
18. Adhikary, S. P., Keshari, N., Urzi, C. and DePhilppis, R., *Algol. Stud.*, 2015, **147**, 67–93.
19. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier, R. Y., *Microbiology*, 1979, **111**(1), 1–61.
20. Nübel, U., Garcia-Pichel, F. and Muyzer, G., *Appl. Environ. Microbiol.*, 1997, **63**(8), 3327–3332.
21. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., *Mol. Biol. Evol.*, 2013, **30**(12), 2725–2729.

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Evidence for underwater current activity on the upper slope of the carbonate platform off western India using multibeam bathymetry

The carbonate platform examined in this study is on the outer continental shelf of the northwestern margin of India at water depths between 60 and 90 m (Figure 1). It is located off the Narmada and Tapi rivers, which debouch abundant terrigenous sediments and freshwater onto the coast. Despite abundant terrigenous material expected on the platform, it comprises <10% terrigenous sediments but abundant carbonate sediments. There may be strong surface or underwater currents preventing deposition of terrigenous material but no evidence for the same has been reported. The platform has been studied since 1971 for single-beam bathymetry, sediments and sedimentary rocks[1–7]. Recent studies on the platform revealed the presence of relict *Halimeda* bioherms, ranging in height from 2 to 20 m, faecal pellets, *Halimeda* and ooid–peloid associated sediments and sedimentary rocks formed during the Early Holocene[6,7]. The importance of *Halimeda* bioherms is that they produce abundant carbonate sediments, and during carbonate production abundant CO_2 is released to the atmosphere[8]. Thus, the study of the growth/demise of bioherms on the platform is helpful to better understand the contemporary climatic and oceanographic conditions. The *Halimeda* bioherms of the Early Holocene and Recent occur in the Indo-Pacific region[9–15]. A few bioherms of the Palaeozoic and Miocene have also been reported[16–18]. *Halimeda* bioherms produce both fine- and coarse-grained carbonate sediments[19–21]. The platform off western India comprises abundant coarse carbonate sediments. The fine-grained sediments (lime muds) produced on the platform were partly deposited in some lagoons and partly transported and deposited on the slope[22–24]. It was assumed that strong bottom currents or tidal currents that prevailed on the platform transported fine-grained carbonates to the slope during the Early Holocene.

Bathymetry data based on widely spaced single-beam echosounder profiles collected on the carbonate platform are available[7]. Single-beam echo-sounding data offer two-dimensional view of the seafloor features, and to know their lateral continuity close-interval data have to be obtained. Multibeam bathymetric data, on the other hand, offer larger aerial view of the morphological features on the seafloor and enable researchers to

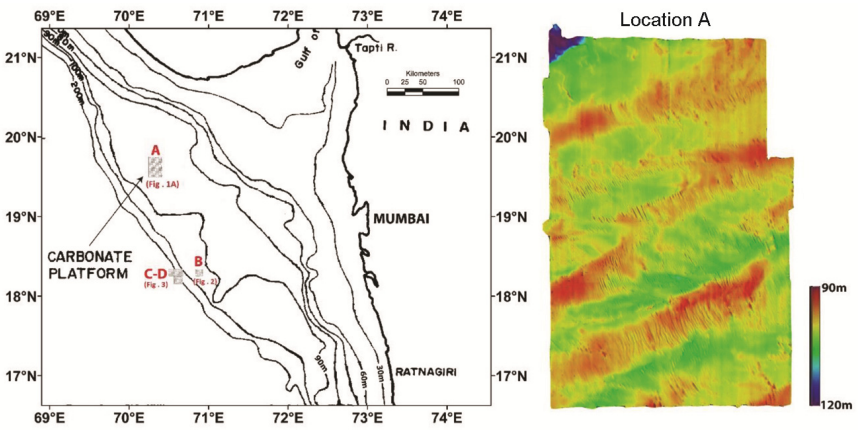

Figure 1. The carbonate platform off western India. (Left) Three boxes (A, B, C-D) shown on the platform are locations where multibeam bathymetry was carried out. (Right) Multibeam bathymetry at location A showing linear ridges and their lateral coalescence, suggesting the presence of algal bioherms.