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Diversity of cyanobacteria in biological crusts on arid soils in the Eastern region of India and their molecular phylogeny

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The biological crusts on lateritic soils, red soils and mine-waste burdened soils in the eastern region of India covering a transect of about 800 km were principally composed of sheathed cyanobacteria of the genera *Scytonema*, *Tolypothrix* and *Lyngbya* along with few other species of *Cylindrospermum*, *Nostoc*, *Calothrix* and *Fischerella.* Molecular phylogeny based on 16S rRNA gene sequence of these cyanobacteria along with those occurring in different habitats of four

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different continents formed a distinct clade, however, were clustered close to other filamentous cyanobacteria.

Keywords: Arid soil, biological crusts, cyanobacteria, molecular phylogeny, morpho-taxonomy.

BIOLOGICAL soil crusts (BSCs) are patch-like structures of different colours that occur on the upper surface of the soil[1]. They represent an association of cyanobacteria, bacteria, chlorophycean algae, fungus and bryophytes forming a film tightly adhering to the soil particles[2]. The relative abundance of these organisms varies according to ecological conditions of the micro-habitat[3]. BSCs occurring in arid and semi-arid zones account for about 30% of the total world landscape[4]; they appear crunchy when dried[5]. In the temperate region mosses and lichens dominate in the crusts, whereas in arid zones where water is a limiting factor, cyanobacteria are found to be the major components along with a few green algal species[4,6]. Cyanobacteria species in soil crusts in the arid zones of India remain dormant during most of the year and appear to form visible biofilms soon after monsoon rainfall[3]. These organisms possess a well-defined polysaccharide sheath layer around their trichome that binds to the soil, thus preventing erosion. They can fix carbon as well as nitrogen and hence play a vital role in the regulation of soil fertility[7]. Although soil crusts are found in almost all types of environment, their distribution has not been extensively studied in the tropics, especially in the Indian subcontinent.

The eastern region of India shows a distinct tropical environment, receiving rainfall only during June–September followed by a prolonged dry spell coupled with high solar irradiance and air temperature up to 45°C for almost 4 months (March–June). Many cyanobacterial species have been reported from the arid regions of the world, e.g. Dead Sea valley and Negev desert, Israel[8,9]; Colorado Plateau, USA[10,11]; Tengger and Ningxia dry land, China[12], and Chadormalu desert, Iran[13]. However, the occurrence of these organisms in the arid soils of India has not yet been analysed. Hence diversity of cyanobacteria in the BSCs covering a transect of about 800 km from West Bengal to Odisha was studied and their molecular phylogeny was determined on the basis of sequence data of similar groups of organism from other parts of the world.

Soil crust samples were collected from three sites in the eastern region of India during July 2011, soon after monsoon rainfall. These were Santiniketan (23°40′53″N, 87°40′22″E) and Salbani (22°37′60″N, 87°19′60″E) in West Bengal, and Bhubaneswar (20°18′45″N, 85°48′48″E) in Odisha (Figure 1). The average annual rainfall at the these sites in 2011 was 125, 160 and 154 cm respectively. Five to ten locations of each site were covered for sampling of BSCs. Samples were dried and kept in the laboratory in closed bottles for analysis. Dried BSCs were soaked and kept in an incubator with fluorescent light of 7.5 W m^{-2} intensity and 25 ± 1°C temperature for 3 days and then analysed. The organisms appeared were inoculated to petri plates containing 1.2% agarized BG 11 ± N medium and purified by repeated sub-culturing. Morphological features such as length, breadth, diameter, etc. were determined using a micrometer, and photographs were taken using a fluorescence microscope with digital camera (Nikon 4500). The species were identified following recent monographs of Komárek and Anagnostidis[14], and Komárek[15].

Genomic DNA was isolated following Kumar and Adhikary[16]. The 16S rRNA gene was amplified using a primer as developed by Nübel *et al.*[17]. The protocols for 16S rRNA gene amplification using PCR and molecular phylogeny analysis were as described earlier[16]. Upon amplification, the fragment length of cyanobacteria from desiccated habitats was always small (not exceeding 500 bp)[16,18,19], which was also reported by other workers[20–22]. The PCR products were purified using Qiagen gel extraction kit and sequenced with the help of GCC Biotech, Kolkata, India.

The sequences generated were deposited in NCBI GenBank. The published gene sequences of cyanobacteria from other parts of the world were retrieved from GenBank through BLAST and used for construction of the phylogenetic tree. The sequence of *Scytonema chiastum* isolated from soil crusts of Tiruchirappalli, Tamil Nadu, South India (S. P. Adhikary, unpublished) was also included. Maximum parsimony method was used for generation of the phylogenetic tree with Mega-4.0 software[23].

A total of 15 cyanobacteria species were isolated from the soil crusts of Santiniketan and Salbani in West Bengal, and Bhubaneswar in Odisha. Each species was

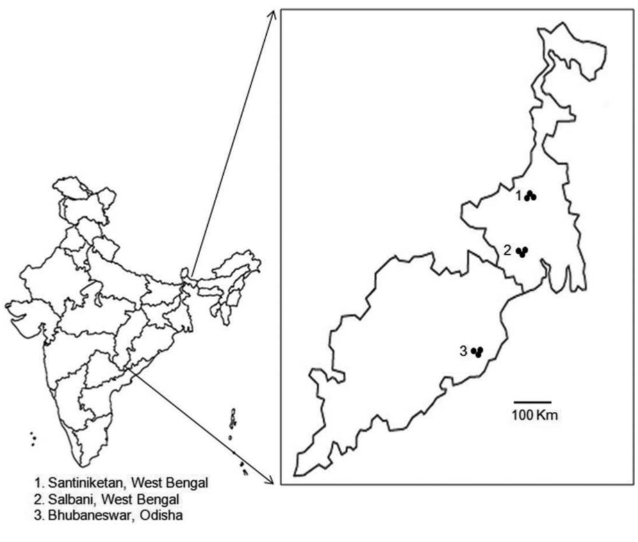

Figure 1. Map of India showing the sampling sites in three different locations of the eastern region.

Table 1. Cyanobacterial species recorded in the biological soil crusts of three different locations: Santiniketan and Salbani in West Bengal and Bhubaneswar in Odisha, eastern India

Cyanobacteria with strain number of the culture	Sampling locations		
	Santiniketan	Salbani	Bhubaneswar
Lyngbya arboricola VB62324	–	–	++
Cylindrospermum majus VB61275	+	–	–
Nostoc microscopicum VB62235	–	–	+
Desmonostoc muscorum VB61269	+	–	–
Nostoc punctiforme VB62229	–	–	+
Nostoc linckia VB61224	–	+	–
Calothrix marchica VB62234	–	–	+
Calothrix elenkini. VB62237	–	–	+
Calothrix bharadwajae VB61272	+	–	–
Calothrix scytonemicola VB61271	+	–	–
Scytonema ocellatum VB61277	+	++	+
Scytonema tolypothrichoides VB61278	++	–	–
Tolypothrix bouteillei VB61268	++	–	–
Tolypothrix fragilis VB61280	+	–	–
Fischerella muscicola VB62230	–	–	+

++, Dominant species appeared soon after wetting of the biological soil crusts (BSCs); +, Associated species appeared upon culture for a prolonged period; –, Absent.

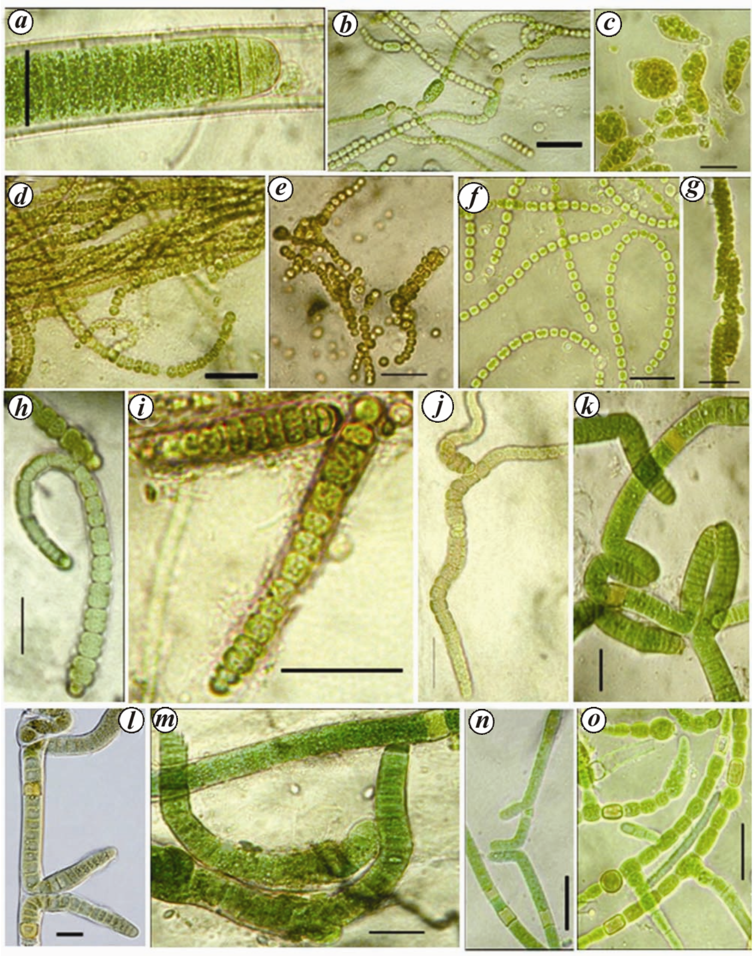

Figure 2. Cyanobacteria isolated from biological soil crusts (BSCs) in Santiniketan (West Bengal), Salbani (West Bengal) and Bhubaneswar (Odisha) and maintained in culture. ***a***, *Lyngbya arboricola*; ***b***, *Cylindrospermum majus*; ***c***, *Nostoc microscopicum*; ***d***, *Desmonostoc muscorum*; ***e***, *Nostoc punctiforme*; ***f***, *Nostoc linckia*; ***g***, *Calothrix marchica;* ***h***, *Calothrix elenkinii;* ***i***, *Calothrix bharadwajae*; ***j***, *Calothrix scytonemicola*; ***k***, *Scytonema ocellatum*; ***l***, *Scytonema tolypothrichoides*; ***m***, *Tolypothrix bouteillei*; ***n***, *Tolypothrix fragilis*; ***o***, *Fischerella muscicola*. Scale bar – 10 μm.

Table 2. Accession number of partial 16S rRNA gene sequences of cyanobacteria retrieved from NCBI GenBank for generating phylogenetic relationship with organisms from the BSCs of the present study

Cyanobacteria	Accession no.	Habitat and place of occurrence	Reference
Fischerella sp. 1711	AJ544076	Soil crust, Papua New Guinea	28
Symphyonema sp. 1269-1	AJ544083	Soil crust, Papua New Guinea	28
Stigonema ocellatum SAG 48.90	AJ544082	Sphagnum bog, New Zealand	28
Phormidium irriguum f. minor	FN813342	Littoral lake, Bratislava, Slovakia	29
Calothrix sp. ANT.L52B.2	AY493625	Antarctic lakes	30
Scytonema sp. DC-A	DQ531704	Soil crust, Colorado Plateau, USA	26
Scytonema sp. FGP-7A	DQ531697	Soil crust, Colorado Plateau, USA	26
Tolypothrix sp. LQ-10	DQ531696	Soil crust, Colorado Plateau, USA	26
Tolypothrix sp. JCT-1	DQ531702	Soil crust, Colorado Plateau, USA	26
Nostoc sp. PCC 8112	AM711537	Laundromat discharge pond, Michigan, USA	31
Nostoc sp. PCC 8976	AM711525	Marshland, Mediterranean coast, France	31
Nostoc sp. TH1S01	AM711547	Rice field, Thailand	31
Rivularia sp. E7 UAM-313	EU009150	Rock surface in river, Spain	32
Rivularia sp. MU15 UAM-369	EU009148	Rock surface in river, Spain	32
Rivularia sp. E1 UAM-302	EU009147	Rock surface in river, Spain	32
Brasilonema octagenarum UFV-OR1	EF150855	*Eucalyptus grandis* leaves, Brazil	33
Fischerella sp. CENA161	EU840724	Freshwater, Sao Paulo, Brazil	34
Nostoc sp. CENA88	GQ259207	Freshwater, Brazil	35
Cylindrospermum sp. PMC238.04	GQ859607	Freshwater, Senegal	36
Cylindrospermum sp. PMC186.03	GQ859606	Freshwater, Senegal	36
Cylindrospermum sp. PMC185.03	GQ859605	Freshwater, Senegal	36
Tolypothrix sp. UAM 335	HM751850	Tejada stream, Spain	37
Stigonema sp. WI53	JQ435860	Freshwater, Wisconsin, USA	38
Scytonema cf. *fritschii* UCFS22	JN565281	Lake bottom, New Zealand	39
Brasilonema tolantongensis	JN676147	Wet limestone wall, Central Mexico	40
Scytonema chiastum VB63238	KC736972	Soil crust, Tiruchirappalli (Tamil Nadu)	Unpublished work of the authors
Tolypothrix bouteillei VB61268	JX523935	Soil crust, Santiniketan (West Bengal)	Present work
Desmonostoc muscorum VB61269	JX523936	Soil crust, Santiniketan (West Bengal)	Present work
Calothrix bharadwajae VB61272	JX523938	Soil crust, Santiniketan (West Bengal)	Present work
Cylindrospermum majus VB61275	JX523939	Soil crust, Santiniketan (West Bengal)	Present work
Scytonema tolypothrichoides VB61278	JX477810	Soil crust, Santiniketan (West Bengal)	Present work
Scytonema ocellatum VB61277	JX523940	Soil crust, Santiniketan (West Bengal)	Present work
Calothrix scytonemicola VB61271	KC736964	Soil crust, Santiniketan (West Bengal)	Present work
Tolipothrix fragilis VB61280	KC736966	Soil crust, Santiniketan (West Bengal)	Present work
Nostoc linckia VB61224	KC736963	Soil crust, Salbani (West Bengal)	Present work
Nostoc punctiforme VB62229	KC736967	Soil crust, Bhubaneswar (Odisha)	Present work
Fischerella muscicola VB62230	KC736968	Soil crust, Bhubaneswar (Odisha)	Present work
Calothrix marchica VB62234	KC736969	Soil crust, Bhubaneswar (Odisha)	Present work
Lyngbya arboricola VB62324	KC924437	Soil crust, Bhubaneswar (Odisha)	Present work
Nostoc microsopicum VB62235	KC736970	Soil crust, Bhubaneswar (Odisha)	Present work
Calothrix elenkinii VB62237	KC736971	Soil crusts, Bhubaneswar (Odisha)	Present work

assigned with a Visva-Bharati culture collection number (VB) that was mentioned along with the 16S rRNA gene sequence accession number against the respective organism (Table 1). These cyanobacteria species belonged to the genera *Lyngbya*, *Cylindrospermum*, *Nostoc*, *Desmonostoc*, *Calothrix*, *Scytonema*, *Tolypothrix* and *Fischerella* (Figure 2). Cyanobacteria belonging to *Scytonema*, *Tolypothrix* and *Lyngbya* appeared soon after monsoon rainfall, and hence were the dominant component in these BSCs. Upon incubation of the BSCs in wet state for prolonged periods, several other species of the genera, *Cylindrospermum*, *Nostoc*, *Desmonostoc*, *Calothrix* and *Fischerella* appeared; hence they were designated as minor organisms. None of these cyanobacteria, except *Scytonema ocellatum* was common to all the three sampling locations from West Bengal and Odisha (Table 1). However, the species commonly occurring in the BSCs in other parts of the world also principally belonged to these genera[6,24–27]. The 16S rRNA gene of only a few of the cyanobacterial species isolated from soil crusts has been sequenced for molecular phylogenetic analysis. These were of the genera *Microcoleus*, *Scytonema* and *Tolypothrix* from Colorado Plateau, USA[26], and *Fischerella* and *Symphyonema* from Papua New Guinea[28]. For molecular phylogenetic relationship on the basis of 16S rRNA partial gene sequences of these organisms in the BSCs from other similar habitats, including freshwaters rock surfaces in rivers, lakes, streams and rice fields of USA, France, Spain, Brazil, New Zealand, Senegal, Thailand, Mexico, Germany, Bratislava and Antarctic, their

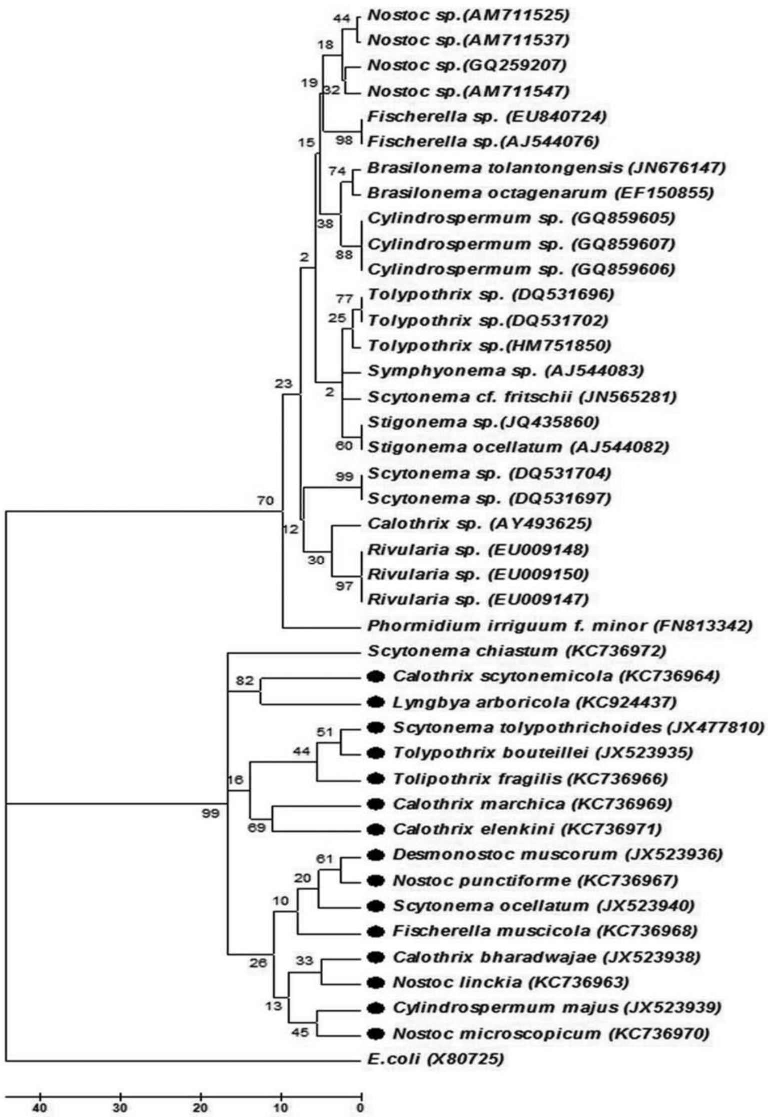

Figure 3. Phylogenetic tree showing the relatedness of 16S rRNA partial gene sequences of the cyanobacteria species isolated from BSCs at Santiniketan and Salbani (West Bengal), and Bhubaneswar (Odisha), eastern region of India, with the species isolated from other habitats at different locations around the world. *Escherichia coli* (X80725) sequence was used as outgroup.

sequences were retrieved from NCBI-GenBank (Table 2). BLAST of these sequences showed that the cyanobacteria species isolated from the BSCs of eastern India clustered together forming a separate clade from those species under the same genera isolated from other regions of the world (Figure 3). The cyanobacteria species with a thick sheath layer around their trichome under the genera *Scytonema*, *Tolypothrix* and *Lyngbya*, which were the dominant component in the BSCs of India, were clustered with filamentous cyanobacteria species isolated from all types of habitats elsewhere. However, a separate clade suggests that these are genetically distant, being acclimatized to monsoonal climate of a tropical environment.

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