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Diversity, molecular phylogeny, and metabolic activity of cyanobacteria in biological soil crusts from Santiniketan (India)

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Abstract The biological soil crusts that appear on the arid soils of Santiniketan, India soon after receiving monsoon rain were studied. The phototrophic organisms in the crusts were principally composed of sheath-forming cyanobacteria belonging to the genera *Scytonema* and *Tolypothrix* as the major components along with associated species of 14 different genera. 16S ribosomal RNA (rRNA) sequencing of these organisms showed that they formed a cluster quite different from *Leptolyngbya*, *Microcoleus*, and *Phormidium*, however, were close to *Scytonema* and *Tolypothrix* reported from similar biofilms on soils of USA and Costa Rica. All these major cyanobacteria species in soil crust possessed scytonemin in higher proportion than chlorophyll *a*, suggesting its role in protection from high solar irradiance and UV. Dried crusts started respiring soon after wetting followed by photosynthesis. Nitrogenase activity also revived after 2 to 12 h of wetting and progressed rapidly with longer period of wet conditions coinciding with appearance of heterocysts in the filaments. These results showed that a number of cyanobacteria with distinct sheath layer survived within the soil crusts in desiccated state and revived their metabolic activity soon after receiving monsoon rain thus contributing to carbon as well as nitrogen fixation in the environment and to nutrient mobilization making the soil productive.

Keywords Biological soil crusts · Cyanobacteria · Molecular phylogeny · Rewetting · Metabolic activity

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Introduction

Biological soil crusts (BSCs) occur on the topmost layer of the soil (Belnap 2003). They appear crusty on the exposed surface of soils in India soon after receiving water on the onset of monsoon rain. They are composed of a variety of microorganisms, e.g., cyanobacteria, green algae, lichens, mosses, microfungi, and other bacteria in different proportions (Belnap and Lange 2001). The photoautotrophic organisms fix carbon in the presence of sunlight, colonize soil surface, and live utilizing available nutrients in the substratum. BSCs principally containing cyanobacteria have been recorded from all biomes in almost all continents covering North America (Rosentreter and Belnap 2001), South America (Büdel 2001a), Europe and Mediterranean region (Büdel 2001b), Arctic Greenland (Hansen 2001), Alps (Türk and Gärtner 2001), Middle East (Galun and Garty 2001), Africa (Ullmann and Büdel 2001; Büdel et al. 2009), Australia (Eldridge 2001), Antarctica (Green and Broady 2001), China (Hu et al. 2003; Zheng et al. 2011), and India (Tirkey and Adhikary 2005, 2006). Most of these organisms possess a well-defined sheath around their trichome and/or copious mucilage which increases soil stability by binding soil particles together, protecting from wind and water erosion (Belnap and Gillette 1998, Zhang et al. 2011). BSCs containing organisms with polymeric matrix of polysaccharides have been reported to influence the soil texture, pore formation, and water retention, which in term determine biological activity in arid lands (Rossi et al. 2012). Also, many of these organisms, being nitrogen fixers, contribute nitrogen to the soil along with carbon, thus playing an important role in mobilization of nutrients in almost all types of soil ecosystems (Belnap et al. 2001; Elbert et al. 2012). Not much information is available on BSCs of Indian subcontinent with a tropical climatic regime, where varying type of soils, ranging from desert in the west to the Alpine in Himalayas along with moist

soils in North-eastern region and heavily eroded soils in the mine belt of central and eastern regions. Crust organisms are only metabolically active when sufficient moisture and light are present and thus sprout onset of rainfall events (Belnap et al. 2004). The monsoon sets in India usually from mid-June to end of August followed by the dry season up to February and then in the hot summer from mid-March to mid-June when they remain dormant. Hence, the precipitation during the monsoon rain has a significant impact on physiological functioning of these organisms in biological soil crust. Previous reports showed that BSCs collected from four different locations in India contained 210–300 mg Chl *a* m^{-2} (Tirkey and Adhikary 2005) with a contribution to carbon fixation comparable to canopy cover in plantation areas (Moore 1998; Copley 2000). Santiniketan, located in West Bengal, in the eastern region of India, is characterized by red soils having poor water-holding capacity and susceptibility to heavy erosion. However, soon after onset of monsoon, during June–July, the soils of the entire region appear green/blackish-green due to cyanobacterial species. In the present work, the cyanobacteria occurring in the BSCs sampled at three different locations in Santiniketan were analyzed following morphometric and molecular approaches. Pigment composition of the BSCs, the sunscreen compounds, and the metabolic activities after wetting were also studied.

Materials and methods

Sampling sites

Soil crust samples were collected using sterile forceps and spatula from five spots each at three locations of Santiniketan in the Birbhum district of West Bengal in Eastern India (from 23° 40′ 53″ to 23° 41′ 33″ N and 87° 40′ 22″ to 87° 40′ 43″ E) during July 2011, within a week after the onset of monsoon rain. The area is located 58 m above the sea level. The annual rainfall during 2011 was 122 cm; however, most of the rain was received during June to September with a peak in July. On the days of sampling, the temperature ranged from 25 to 32 °C, humidity from 69 to 93 %, and rainfall 6 to 21 mm at the collection sites. The samples were air dried and stored in specimen tubes for further analysis. The BSCs were soaked with sterile distilled water, incubated under fluorescent light at 7.5 W m^{-2} up to 72 h, and examined intermittently. The organisms that appeared were transferred to agar plates prepared with BG 11±N medium (Rippka 1979), cultured at 25±1 °C, and subsequently purified following Kaushik (1987). The organisms which appeared within 72 h of wetting were considered as the major component in the BSCs, and those which occurred in the culture along with the major organisms upon prolonged incubation in liquid medium up to 1 month were designated as associated organisms. Measurement of length, breadth, and diameter of the cells of each organism was carried out using stage and ocular micrometers, and microphotographs were taken using an Olympus BX41 microscope equipped with Nikon 4500 Coolpix digital camera. The cyanobacterial species were identified following Desikachary (1959), Komárek and Anagnostidis (1989, 1999, 2005), and Anagnostidis and Komárek (1990).

DNA isolation and PCR

Genomic DNA was extracted from cyanobacterial cells following bacterial genomic DNA isolation protocol. Exponentially growing cells were pelleted by centrifugation and then resuspended in 567 μL lysis buffer (10 mM Tris–HCl, pH 8.0, 1 mM trisodium citrate, and 1.5 % sodium dodecyl sulfate (SDS)) followed by incubation for 1 h at 37 °C after addition of 30 μL of 10 % SDS and 3 μL of 20 mg mL^{-1} proteinase K. To this, 100 μL 5 M NaCl was added, followed by addition of 80 μL CTAB/NaCl solution (10 % CTAB/0.7 M NaCl), and incubated for 10 min at 65 °C. The lysate was extracted with equal volume of chloroform/isoamyl alcohol (24:1) and then equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). The DNA was precipitated with 0.6-volume isopropyl alcohol, washed with 70 % ethanol, air dried, and resuspended in 100 μL TE buffer. PCR amplification of 16S ribosomal RNA (rRNA) gene of the experimental organisms was carried out using CYA359F and CYA781R (equimolar mixture of CYA781R-a and CYA781R-b) primers for cyanobacteria (Nübel et al. 1997). Template DNA (10 ng) was added to the reaction mixture of total 50 μL containing 31 μL milliQ water, 5 μL 10× Buffer (15 mM $MgCl_2$), 2 μL dNTPs (10 mM), 2.5 μL forward primer CYA359F, 1.25 μL each of reverse primers CYA781R-a and CYA781R-b, 1 μL Taq polymerase, and 1 μL BSA (20 μg μL^{-1}). Amplification was done using the PCR system 9700 (Applied Biosystems). The products were purified using Qiagen gel extraction kit and sequenced (GCC Biotech, India).

Extraction and quantification of chl *a*, carotenoids, and scytonemin

Fifty mg of air-dried soil crust was taken, and pigments were extracted in 90 % (*v*/*v*) methanol. Chl *a* and carotenoids were quantified by following the method of Mackinney (1941) and Davis (1976), respectively. Scytonemin pigment from the BSCs and the corresponding major cyanobacterium in pure culture were extracted in acetone (100 %) and quantified (Garcia-Pichel and Castenholz 1991). Absorption spectra of 90 % (*v*/*v*) methanolic extract of the BSCs as well as the corresponding cyanobacteria from culture was also taken in

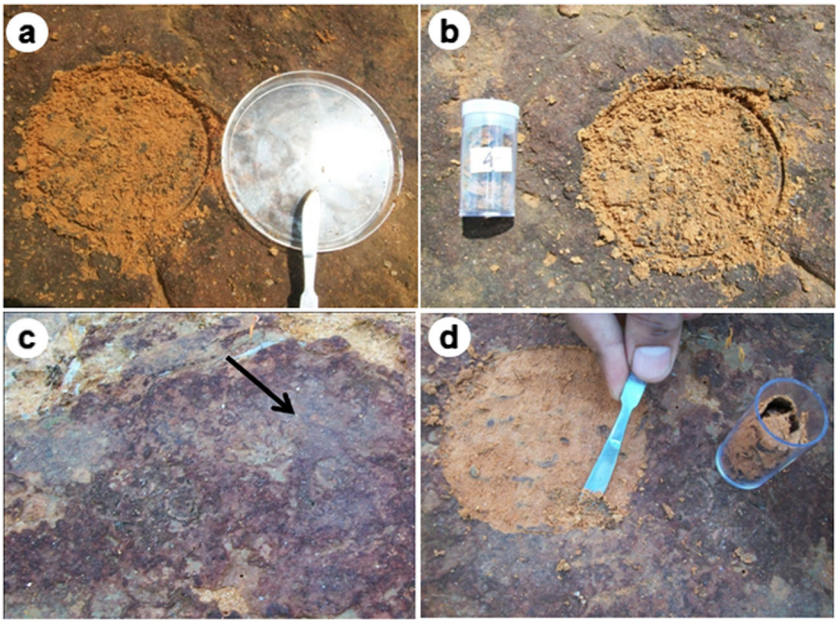

Fig. 1 Photograph showing the formation of biological soil crusts (BSCs) soon after receiving monsoon rain at three different locations in Santiniketan in West Bengal, India: **a** Siksha Bhavana; **b** Deer Park; and **c**, **d** Khoai

the wavelength range of 250–750 nm to determine the ratio of different photosynthetic as well as UV-absorbing pigments. Absorption spectra were taken in a Shimadzu 1800 UV-vis spectrophotometer using quartz cuvettes.

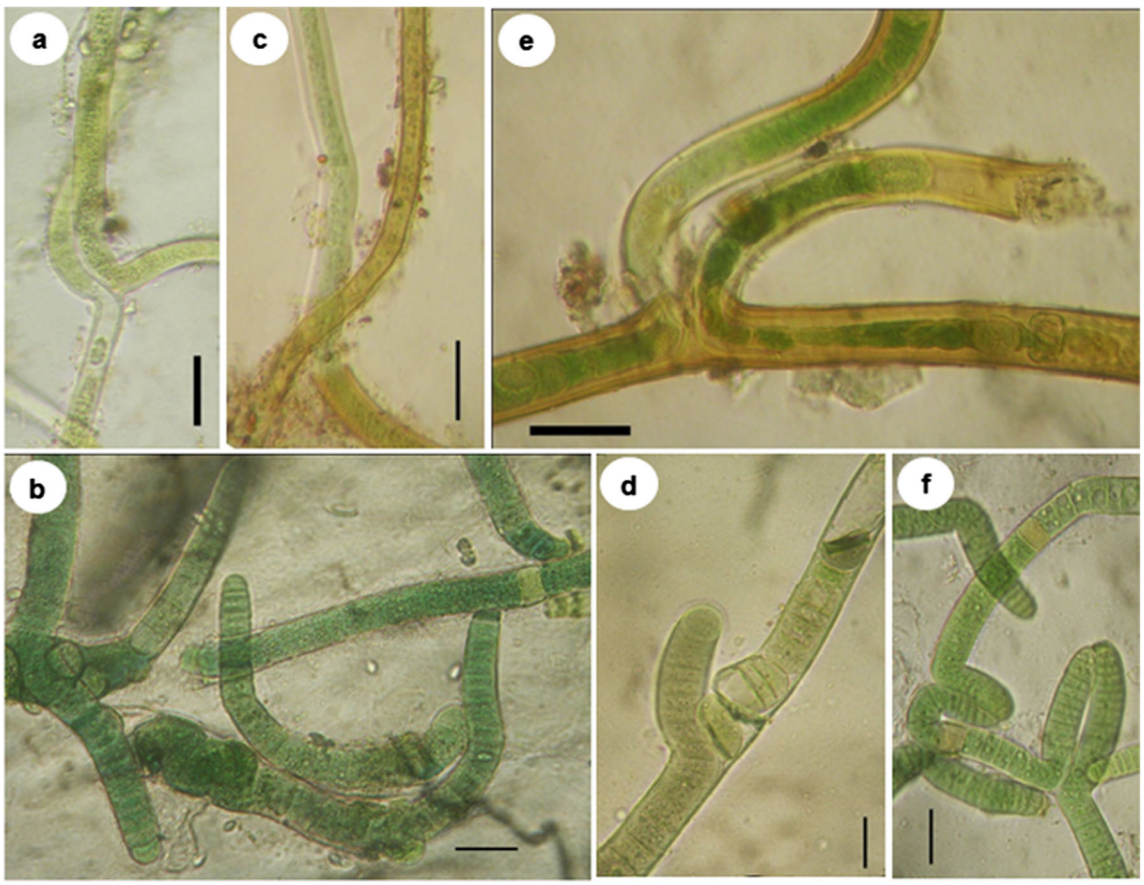

Fig. 2 Major cyanobacterial species isolated from BSCs of Santiniketan: Siksha Bhavana, **a** appeared within 24 h of wetting BSCs and **b** *Tolypothrix bouteillei* in culture of the same soil crust; Deer park, **c** appeared within 24 h of wetting; **d** *Scytonema tolypothrichoides* in the culture of the same soil crust; Khoai, **e** appeared within 24 h of wetting of BSC, **f** *Scytonema ocellatum* in culture of the same soil crust (*scale bars*= 10 μm)

Measurement of photosynthetic and respiratory activities

Photosynthetic and respiratory activities of soil crust as well as of the corresponding major cyanobacterium in pure culture were measured using a Clark-type oxygen electrode (Hansatech, UK). A 100-mg soil crust sample was soaked in distilled water and transferred to the DW-1 reaction vessel having an outer jacket for water circulation to maintain the temperature at 25 °C. Suspension was stirred with magnetic stirrer. Light source was provided from a Xenophot 100 W halogen lamp. A preamplifier was used to amplify the signal generated from the cathode to increase the efficiency of recording in a strip chart recorder. Photosynthetic and respiratory activities were expressed as μmol O_2 g^{-1} dried material h^{-1}.

Acetylene reduction activity

For the measurement of nitrogenase activity through acetylene reduction assay, 1 g of soil crust as well as of dried cells of the pure culture of the corresponding major cyanobacteria was taken in 10-mL glass vials, and BG11 medium was added in each tube to make the volume up to 7.5 mL. All the vials were capped airtight with silicon septa and incubated under fluorescent light. Acetylene gas was injected into the vials providing up to 10 % gaseous atmosphere. At intervals of 2, 12, 24, and 48 h, 100 μL of gas phase was withdrawn from each vial with airtight Hamilton syringe and then analyzed for ethylene in AIMIL-Nucon 5765 gas chromatograph with FID detector fitted with Porapak-T SS column (80–100 mesh; carrier gas nitrogen with flow rate 30 mL min^{-1}; column temperature—100 °C; injector temperature—110 °C; detector temperature—120 °C). Acetylene reduction activity was expressed as nmol ethylene g^{-1} air-dried material h^{-1}.

Results

BSCs showing various colorations on the soil surface at different locations of Santiniketan e.g., dark green (Siksha Bhavana), light green (Deer park), and reddish-brown (Khoai) were collected and analyzed for the organisms appearing within 24 h of wetting and thereafter up to 72 h (Fig. 1). These cyanobacterial species belonging to the genera *Tolypothrix* and *Scytonema* appeared in the BSCs soon after wetting were designated as the major organisms in the crust (Fig. 2, Table 1). Upon prolonged wetting of the BSCs, several cyanobacterial species appeared as associated species to the major component. These belonged to the genera *Gloeocapsa, Asterocapsa, Chroococcidiopsis, Phormidium, Pseudophormidium, Porphyrosiphon, Spirulina, Fortiea, Cylindrospermum, Nostoc, Calothrix, Schizothrix, Scytonema,* and *Tolypothrix* (Table 1). The 16S rRNA sequences of three major cyanobacteria species in the BSCs of Santiniketan generated in this study were deposited in the GenBank under accession numbers JX523935, JX477810, and JX523940. Phylogenetic tree was constructed by taking the 16S rRNA sequences of these three species along with several other cyanobacteria from similar habitats reported earlier following neighbor-joining method using MEGA 4.0 software (Tamura et al. 2007). Details of these cyanobacteria isolated from BSCs from other locations/regions and their 16S rRNA sequence accession numbers retrieved from GenBank are given in Table 2 and also referred in the dendrogram

Table 1 Occurrence of major and associated cyanobacterial species in the biological soil crusts (BSCs) from three different locations in Santiniketan, West Bengal, India

Organism	Sampling site		
	Siksha Bhavana	Deer park	Khoai
Major organisms (appeared within 24 h of wetting of BSCs)			
Tolypothrix bouteillei	+++		
Scytonema tolypothrichoides		+++	
Scytonema ocellatum			+++
Associated organisms (appeared upon prolonged wetting of BSCs)			
Gloeocapsa compacta	+		
Asterocapsa divina	+		
Chroococcidiopsis kashayi	+		
Phormidium chlorinum	+		
Phormidium borianum		+	
Phormidium bulgaricum		+	
Phormidium tergestinum		+	
Phormidium incrustatum		+	+
Pseudophormidium flexuosum	+		
Pseudophormidium hollerbachianum	+		
Porphyrosiphon fuscus	+		
Spirulina tenerrima		+	
Fortiea caucasica	+		
Cylindrospermum majus		+	
Nostoc microscopicum		+	
Nostoc muscorum	+		
Nostoc punctiforme	+		+
Calothrix bharadwajae		+	+
Calothrix scytonemicola	+		
Schizothrix lardacea	+		
Scytonema bewsii	+		
Scytonema mirabile		+	
Tolypothrix fragilis		+	
Tolypothrix rechingeri	+		
Tolipothrix robusta		+	

+++ dominant species in the naturally occurring BSCs, + minor component in culture

Table 2 16S rRNA sequence information of soil crust cyanobacteria retrieved from NCBI GenBank for comparison

Organism	Accession no.	Place and country	Reference
Microcoleus vaginatus	AF284803	Colorado Plateau, USA	Garcia-Pichel et al. 2001
Microcoleus steenstrupii	AF355379	Mojave, USA	Boyer et al. 2002
Microcoleus steenstrupii	AF355396	Mojave, USA	
Microcoleus steenstrupii	AF355394	Chihuahuan, USA	
Microcoleus steenstrupii	AF355392	Great Basin, USA	
Microcoleus steenstrupii	AF355384	Great Basin, USA	
Phormidium sp. OL M10	AM398791	Mellum Island, Germany	Marquardt & Palinska 2007
Phormidium sp. OL S3	AM398785	Mellum Island, Germany	
Phormidium sp. OL 05	AM398798	Mellum Island, Germany	
Phormidium sp. OL S5	AM398787	Mellum Island, Germany	
Phormidium sp. OL 32	AM398793	Mellum Island, Germany	
Phormidium sp. AA	AM398777	Nizzara, Israel	
Phormidium foveolarum SAG 1462-1	AM398778	Faridpur, India	
Phormidium autumnale CCAP1462/10	AM398804	South Orkney, Antarctica	
Phormidium animale CCALA140	AM398799	Crater of volcano, Italy	
Scytonema sp. FGP-7A	DQ531697	Colorado Plateau, USA	Yeager et al. 2007
Scytonema sp. FGP-7A	DQ531698	Colorado Plateau, USA	
Scytonema sp. DC-A	DQ531704	Colorado Plateau, USA	
Scytonema sp. DC-A	DQ531701	Colorado Plateau, USA	
Tolypothrix sp. LQ-10	DQ531696	Colorado Plateau, USA	
Tolypothrix sp. JCT-1	DQ531702	Colorado Plateau, USA	
Scytonema sp.	EU818967	Costa Rica, Las Cruces	Lücking et al. 2009
Desertifilum tharense	FJ158995	Thar Desert, India	Dadheech et al. 2012
Desertifilum tharense	FJ158996	Thar Desert, India	
Desertifilum tharense	FJ158997	Thar Desert, India	
Tolypothrix bouteillei	JX523935	Santiniketan, India	Present work
Scytonema tolypothrichoides	JX477810	Santiniketan, India	
Scytonema ocellatum	JX523940	Santiniketan, India	

(Fig. 3). Bootstrap values at nodes were obtained from 1,000 replicates. *Escherichia coli* (X80725), a non-cyanobacterial sequence was used as the out-group (Cilia et al. 1996).

Chlorophyll *a*, carotenoid, and scytonemin pigment contents of equal amount of BSCs from the three locations in Santiniketan were determined (Table 3). It showed that the colonization of cyanobacteria in the sandy and sand-clay mixture were more intense, resulting in much higher chlorophyll *a* and carotenoid contents ranging from 105 to 148 and 4.4 to 8.0 μg g^{-1} soil crust, respectively. BSCs on sandy-gravel soil showed the least content of chlorophyll *a* as well as carotenoids (Table 3). Absorption spectra of methanolic extract (90 % *v*/*v*) of the BSCs and their respective major organism were also taken at the wavelength range of 250 to 750 nm (Fig. 4). The extracts of all the three different BSCs showed prominent absorption at 665 nm due to chl *a*, at 504 and 470 nm due to carotenoids, and also at 384 nm due to scytonemin pigment. Absorption spectra of corresponding major cyanobacterium in culture retained absorption due to scytonemin as well as carotenoids at 470 nm, but the pigment absorbing at 504 nm was not detected. Further, the quantity of carotenoids and scytonemin with respect to chl *a* pigment of the BSCs was much higher in comparison to those in culture of the respective organism (Fig. 4, Table 4).

All the three BSCs absorbed water rapidly soon after wetting. *Tolypothrix bouteillei,* the major organism in the crust on sandy-clay soil started its respiration as well as photosynthesis within 15 min of wetting, and these processes reached optimum activity almost within 24 h (Table 5). In the BSCs from sandy-gravel and sandy soil harboring *Scytonema* species, the photosynthesis was delayed from 15 to 30 min after wetting. However, both showed respiratory activity within few minutes of rewetting. These anabolic and catabolic rates of all the three organisms increased with further duration of wetting and became nearly saturated in 48 h with the ratio of their photosynthesis to respiration ranging from 1.59 to 3.06. Almost similar ratios of both the processes were obtained in pure culture of the organisms varying from 1.82 to 3.76 (Table 5). Similarly, nitrogenase activity of the BSCs from sandy-clay soil was revived within 2 h of wetting and those

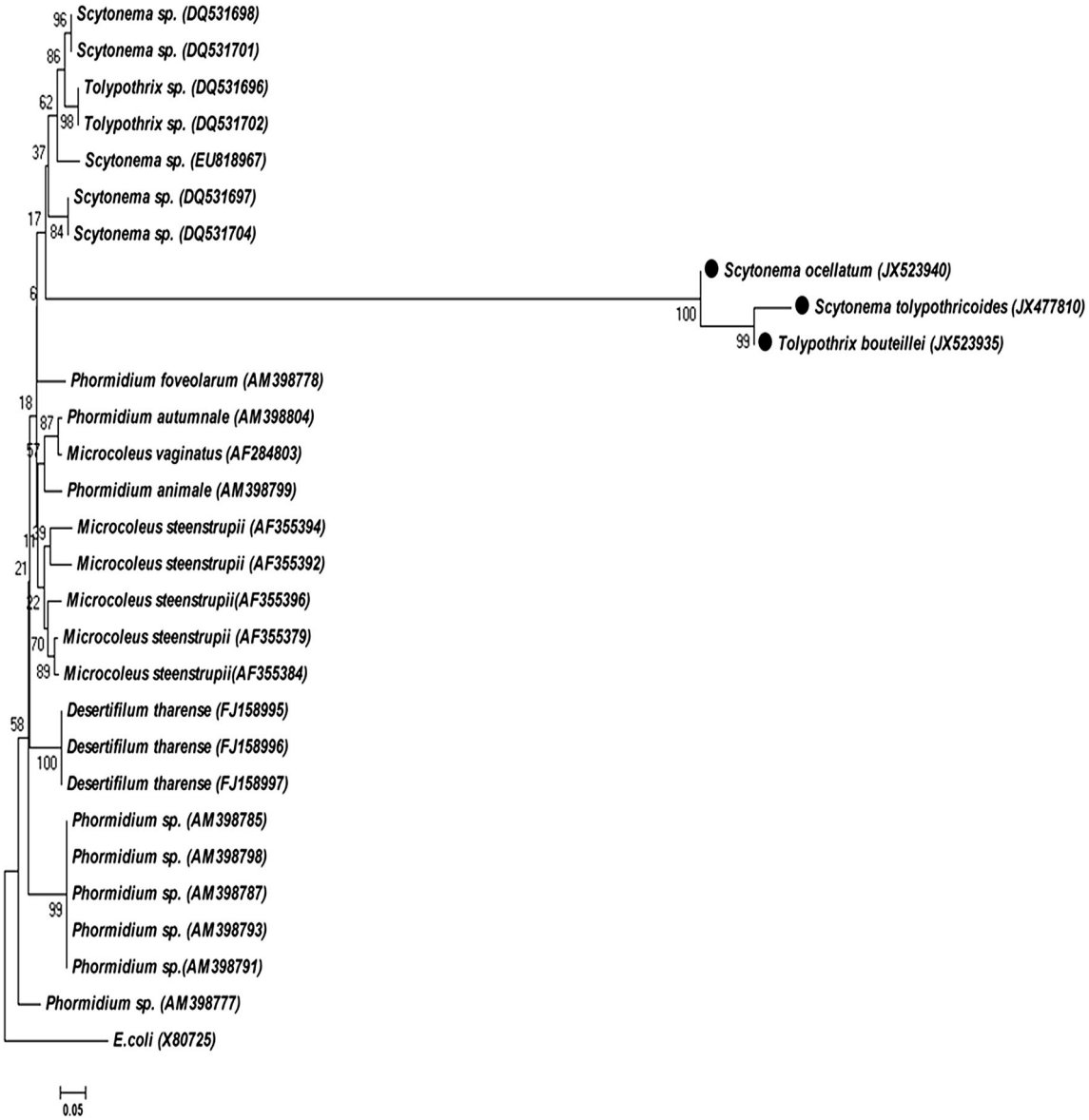

Fig. 3 Tree showing the phylogenetic relatedness of 16S rRNA gene sequence of major cyanobacterial species from BSCs of Santiniketan, India with the species from similar habitats in others locations of the globe. Tree was generated through neighbor joining using MEGA-4.0 software

from sandy-gravel and sandy soil were delayed up to 12 h. Acetylene reduction activity (ARA) of all the three BSCs progressed steadily thereafter with further duration of wetting. ARA after 48 h of wetting of the BSCs were 158, 126, and 93 nmol C_2H_4 g^{-1} material h^{-1} from sandy-clay, sandy-gravel, and sandy soils, respectively. The ARA of the corresponding

Table 3 Chlorophyll *a*, carotenoid, and scytonemin pigment content in one gram soil crust sample from different places in Santiniketan, West Bengal, India

Places of collection	Type of soil	Major organism in the BSCs	Chl *a* (μg g^{-1} soil crust)	Carotenoid (μg g^{-1} soil crust)	Scytonemin (μg g^{-1} soil crust)	Chl *a* (mg m^{-2} area)
Siksha Bhavana	Sandy-clay mixture	*Tolypothrix bouteillei*	105±8.2	4.4±0.5	1,015±50.8	50.8±3.2
Deer park	Sandy-gravel	*Scytonema tolypothrichoides*	15±1.4	0.7±0.1	45±5.2	13.7±1.1
Khoai	Sandy	*Scytonema ocellatum*	148±12.1	8.0±0.7	936±65.9	104±9.2

Value represents mean of three independent determination±S.D.

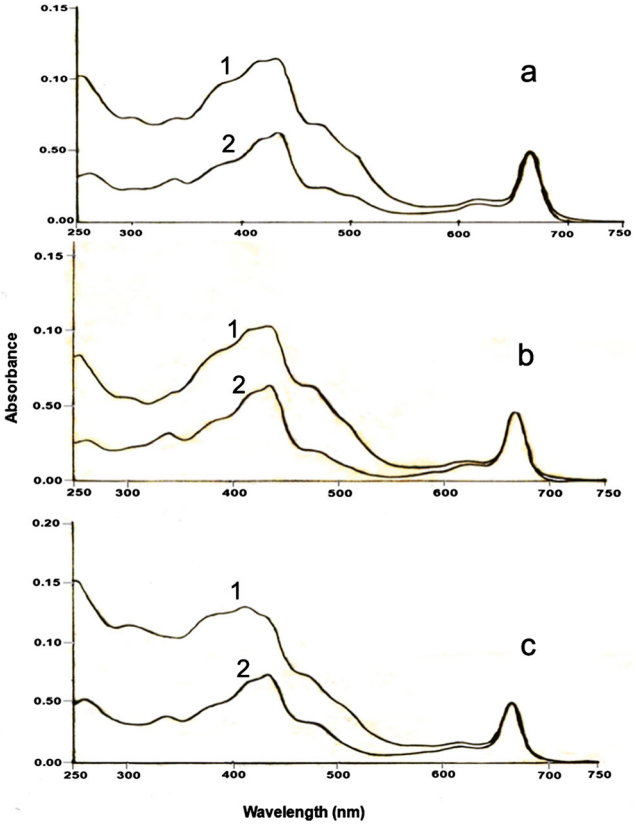

Fig. 4 Absorption spectra of 90 % (*v*/*v*) methanolic extract of **a** *Tolypothrix bouteillei*: *1* in BSCs of Siksha Bhavana, *2* in pure culture; **b** *Scytonema tolypothrichoides*: *1* in BSCs of Deer park, *2* in pure culture; **c** *Scytonema ocellatum*: *1* in BSCs of Khoai, *2* in pure culture

major cyanobacterium in pure culture in full grown state was 245, 187, and 144 nmol C_2H_4 g^{-1} material h^{-1} (Table 6). Microscopic examination of wetted crusts showed that heterocysts appeared in the filaments within 2 to 12 h of wetting, thus initiating the ARA when the organisms became metabolically reactivated.

Discussion

Analysis of these results showed that none of the major cyanobacteria was common to the BSCs sampled from different locations in Santiniketan, in the eastern region of India. *T. bouteillei* was the principal organism in the BSCs of Siksha Bhavana on sand-clay mixed soil, imparting dark green coloration to the substratum, whereas *Scytonema tolypothrichoides* was the main component in the BSCs of Deer park with sandy-gravel substratum showing light green color. *Scytonema ocellatum* with a thick-colored sheath imparted reddish-brown coloration to the sandy soils of Khoai due to their appearance soon after receiving monsoon rain. Species belonging to the genera *Gloeocapsa, Asterocapsa, Chroococcidiopsis, Pseudophormidium, Porphyrosiphon, Spirulina, Fortiea, Cylindrospermum,* and *Schizothrix* which occurred in the BSCs of Santiniketan have not been reported earlier in other regions of India (Tirkey and Adhikary 2005, 2006). These associated cyanobacterial species were not the same in all the BSCs from three different locations in Santiniketan except for the *Nostoc punctiforme*, which was common to Siksha Bhavana, and Khoai, while *Phormidium incrustatum* and *Calothrix bharadwajae* were common to Deer park and Khoai area. The earlier report on BSCs in different regions of India comprising laterite soil of Bhubaneswar, brown forest soil of Salbani, arid soil of Tiruchirappalli, and sandy soil of Goa showed that the principal organisms were *Lyngbya arboricola, S. ocellatum, Scytonema chiastum,* and *Plactonema notatum,* respectively, and the associated organisms were different species of *Nostoc, Lyngbya, Oscillatoria, Plectonema, Microcoleus, Calothrix, Anabaena, Scytonema, Tolypothrix, Westiellopsis, Fischerella, Hapalosiphon,* and *Stigonema* (Tirkey and Adhikary 2005, 2006). Invariably, species of *Lyngbya, Scytonema, or Tolypothrix* were the major component in the soil crust of India irrespective of the locations.

The composition of cyanobacteria in the BSCs in other regions of the globe showed the occurrence of 22, 13, 12, 11, 10, 8, 7, 6, 6, 5, 4, 3, 3, and 2 species in the genera *Nostoc, Scytonema, Phormidium, Microcoleus, Oscillatoria, Schizothrix, Leptolyngbya, Tolypothrix, Chroococcus, Lyngbya, Chroococcidiopsis, Aphanothece, Calothrix,* and *Aphanocapsa,* respectively, and 1 species each of the genera *Microcystis, Anabaena, Plectonema, Porphyrosiphon, Scynechococcus, Nostocopsis, Chlorogloea, Petalonema,*

Table 4 Absorbance of 90 % (*v*/*v*) methanolic extract of three different BSCs and their respective major organisms at different wavelengths corresponding to Chl *a* (665 nm), carotenoids (504 and 470 nm), and scytonemin pigment (384 nm)

Places of collection	Major organism in the BSCs	Absorption at 384 nm	Absorption at 470 nm	Absorption at 504 nm	Absorption at 665 nm	Ratio of absorbance 384:470:504:665
Siksha Bhavana crust	*T. bouteillei*	0.099	0.071	0.051	0.05	1.98:1.42: 1:1
		0.041	0.024		0.05	0.82:0.48 :1
Deer park crust	*S.tolypothrichoides*	0.087	0.066	0.044	0.05	1.74:1.32:0.88:1
		0.040	0.020		0.05	0.8:0.4:1
Khoai crust	*S. ocellatum*	0.129	0.079	0.055	0.05	2.58:1.58:1.1:1
		0.048	0.034		0.05	0.96:0.68 :1

Table 5 Photosynthetic and respiratory activity (μmol O_2 g^{-1} material h^{-1}) of BSCs from three locations in Santiniketan after different durations of wetting and of the corresponding cyanobacteria in culture

Places of collection	Different duration of wetting									Corresponding major organism in BSCs
	15 min	30 min	1 h	2 h	4 h	8 h	16 h	24 h	48 h	
Siksha Bhavana										*T. bouteillei*
Photosynthesis	2.4±0.6	5.9±1.1	8.3±2.4	21.2±1.8	35.8±4.2	54.9±4.6	72.4±8.1	98.6±8.7	103±9.8	122±5.6
Respiration	3.3±0.9	5.6±1.3	8.8±3.4	17.2±5.4	32.3±3.9	46.7±5.8	55.2±7.3	61.3±9.2	64.5±8.7	67±5.3
Deer park										*S. tolypothrichoides*
Photosynthesis	0	3.8±0.5	5.7±0.5	13.1±1.2	26.9±2.8	48.2±5.5	67.9±4.7	94.1±8.4	116.8±11.2	186±9.8
Respiration	5.1±0.2	8.3±2.7	11.2±0.7	16.9±3.3	22.8±3.7	28.7±2.4	35.4±3.2	42.9±4.1	47.2±7.6	55±4.2
Khoai										*S. ocellatum*
Photosynthesis	0	0	3.0±0.2	12.6±0.9	21.5±1.3	33.2±2.6	54.8±3.5	76.7±3.9	112.0±9.3	162±7.5
Respiration	3.9±0.6	6.2±0.8	7.9±0.7	9.2±0.7	15.2±0.9	23.5±1.2	28.2±1.4	33.7±0.8	36.5±2.2	43±3.4

Value represents mean of three independent determination±S.D.; for each experiment, equal amount of BSCs was used

Pleurocapsa, Xenococcus, Myxocarcina, Stigonema, Cyanothece, Pseudophormidium, Trichocoleus, and *Trichormus* (Jeanfils and Tack 1992; Johansen 1993; Dor and Danin 1996; Ullmann and Büdel 2001; Hu et al. 2003; Tell and Mataloni 2005; Alwathnani and Johansen 2011). Of these, only seven species of *Nostoc*; five species of *Scytonema;* three species each of *Chroococcus, Phormidium, and Lyngbya;* two species each of *Oscillatoria and Calothrix*; and one species each of *Plectonema* and *Microcoleus* were common with those reported in the BSCs of India (Madhusoodanan and Dominic 1996; Bhatnagar and Bhatnagar 2005; Tirkey and Adhikary 2005, 2006; Bhatnagar et al. 2008). The BSCs of temperate region from Europe and USA showed occurrence of a number of green algae e.g., *Zygogonium ericetorum, Klebsormidium flaccidum, Klebsormidium mucosum, Stichococcus bacillaris,* and *Stichococcus* sp. as well as mosses e.g., *Polytrichum piliferum, Cephaloziella* sp., *Brachythecium albicans, Ceratodon purpureus, Polytrichum piliferum, Hypnum jutlandicum,* and *Pleurozium schreberi* (Belnap et al. 2001; Büdel 2001a; Türk and Gärtner 2001) which did not occur in the BSCs on the soil of different locations in India so far reported. These results showed that the climatic and microclimatic regimes of a tropical region like Eastern India, receiving monsoon only during certain time of the year (July–September) followed by prolonged dry period (February–June) coupled with high soil temperature, govern the occurrence of some specific cyanobacterial species with distinct sheath layer on their trichome as the major component and other spore-forming cyanobacterial species as the associated forms.

Molecular phylogenetic analysis using 16S rRNA sequence of the three major cyanobacterial species *T. bouteillei* VB61268, *S. tolypothrichoides* VB61278, and *S. ocellatum* VB61277 from the soil crust of Siksha Bhavana, Deer park, and Khoai, respectively, along with 25 other cyanobacterial species isolated from soil crusts across the globe showed that all the major cyanobacteria belonging to *Scytonema* and *Tolypothrix* from BSCs of Santiniketan formed a distinct cluster close to species under the same genera from BSCs of Colorado Plateau, USA and Costa Rica (Yeager et al. 2007; Lücking et al. 2009). However, in the tree, these were placed distinctly apart from the genera *Phormidium*, *Microcoleus*, and *Desertifilum* from soil crusts of Germany, Israel, Italy, and Antarctica (Garcia-Pichel et al. 2001; Boyer et al. 2002;

Table 6 Acetylene reduction activity (ARA nmol C_2H_4 g^{-1} material h^{-1}) of BSCs from three places in Santiniketan after different durations of wetting in light and their corresponding cyanobacteria from culture

Places of collection	Different durations of wetting					Corresponding major organism in BSCs
	0 h	2 h	12 h	24 h	48 h	
Siksha Bhavana	0	5.4±0.8	18.6±1.3	96±5.8	158±11.7	*T. bouteillei* 245±12.3
Deer park	0	0	3.8±0.9	22.5±1.8	126±14.2	*S. tolypothrichoides* 187±16.9
Khoai	0	0	5.2±0.7	12.8±1.2	93±8.6	*S. ocellatum* 144±12.7

Value represents mean of three independent determination±S.D.; for each experiment, equal amount of BSCs was used

Marquardt and Palinska 2007; Dadheech et al. 2012) (Table 2, Fig. 3).

The chlorophyll *a* m^{-2} in the BSCs at all the three locations of Santiniketan showed a distinct variation. The quantity was 104 mg m^{-2} in sandy soil followed by sand-clay mixture (50.8 mg m^{-2}) and sandy-gravel soil (13.7 mg m^{-2}), showing that quantity of photosynthesizing cells in the BSCs was also governed by the type of soil on which they occur. Previous reports on the quantity of chl *a* g^{-1} crust in different regions of India showed that it ranged from 248 to 282 mg m^{-2} (Tirkey and Adhikary 2005). These results indicated that in the red soil of Santiniketan, with poor water holding capacity, the coverage of BSCs was less with a lower amount of chl *a* m^{-2}. The BSCs harboring *Scytonema* as the major component looked either green or reddish-brown in color due to presence of higher quantity of carotenoids and scytonemin pigments. There are several reports that sheath of cyanobacteria harbors UV sunscreen pigments, especially scytonemin absorbing at 384 nm, and provides protection to cells from injury due to UV radiation (Büdel et al. 1997; Roy et al. 1997; Adhikary and Sahu 1998; Dillon and Castenholz 1999). Also, Fleming and Castenholz (2007) reported periodic desiccated strains of *N. punctiforme* and *Chroococcidiopsis* sp. produced higher amount of scytonemin when exposed to UV-A, suggesting that the pigment reduce UVR damage to metabolically inactive cells. In the present work, the three major organisms in the BSCs were sheath forming and colored due to presence of carotenoids and scytonemin, thus enabling them to survive in desiccated state even under prolonged exposure to high solar insolation of the tropics.

Certain species of sheath-forming cyanobacteria have been reported to survive in soils under drought conditions for several years because of their capability to withstand desiccation and high temperature (Tomaselli and Giovannetti 1993). It has been shown that the terrestrial cyanobacteria *Nostoc flagelliforme* as well as *Nostoc commune* started respiring within few minutes of rewetting simultaneously evolving oxygen due to recovery of photosynthetic system, showing that they were metabolically dormant under dry state and became active after receiving moisture (Scherer et al. 1984; Satoh et al. 2002; Chen et al. 2011). In the present work, the BSCs from all the three different types of soil showed respiratory activity soon after wetting. In the sandy-clay soil of Siksha Bhavana, the organism in the BSCs evolved oxygen within 15 min of wetting which was delayed up to 30 min in the sandy-gravel soil of Deer park and for up to 1 h in the sandy soils of Khoai. Oxygen uptake as well as evolution increased thereafter in all the BSCs of three different soils, and the ratio of photosynthesis to respiration was stabilized within 48 h. The maximum values of the ratio of these two metabolic activities were 1.82, 3.38, and 3.76 in pure culture of the corresponding major organism in the BSCs, *T. bouteillei, Scytonema tolypothrichoids,* and *S. ocellatum,* respectively, which was nearly the same value obtained in the rewetted soil crusts up to 48 h. There are reports that upon rewetting of cyanobacteria biofilms from subaerial habitats, the initiation of photosynthesis was delayed up to 1 h, and even after prolonged incubation in light, the ratio of photosynthesis to respiration was nearly one resulting in extreme slow growth of the organisms (Keshari and Adhikary 2013). To the contrary, the cyanobacteria in the BSCs started photosynthesizing within few minutes of wetting and revived its fullest activity within 48 h, showing that they could survive in dormant state in the arid climatic regime of the tropics during summer months and revived their metabolic activity soon after receiving monsoon rain. Scherer et al. (1984) have shown that even after keeping the terrestrial cyanobacterium *N. commune* in dry state for 2 years, its nitrogenase activity was revived and maximum nitrogen fixation value was recorded in 120–150 h of wetting. However, nitrogenase activity of the BSCs from sandy-clay soils of Santiniketan was revived after 2 h and in the other two soil types within 12 h of wetting. The rate of nitrogen fixation increased further with the duration of wetting and was closely corroborated with the appearance of heterocysts in the filaments. These results showed that the cyanobacteria in the BSCs in a tropical country like India remained in the dormant state during desiccation period and had the potentiality to revive within few hours of receiving monsoon rain.

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