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Cyanobacterial flora from Sidpur geothermal spring, Jharkhand, India – First report

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Abstract

This is the first report of species diversity of the cyanobacterial component in the biological mat of an alkaline thermal spring at Sidpur located in the district Pakur of Jharkhand state, India. The cyanobacterial species are both thermotolerant and thermophilic based on temperature tolerance. Distinct four types of mats were recognized dominated by: i) *Leptolyngbya*, ii) *Phormidium,* iii) Thermophilic *Synechococcus* and iv) *Mastigocladus* respectively. Sixteen species belonging to eleven genera of cyanobacteria were identified among which *Leptolyngbya* exhibited widespread distribution along the thermal gradient. Besides, *Thermosynechococcus*, Thermophilic *Synechococcus, Chroococcus, Gloeocapsa, Phormidium*, *Oscillatoria, Desmonostoc, Nostoc, Chlorogloeopsis* and *Mastigicladus* were isolated using BG-11 medium and maintained at a light intensity of 25–30 µmol photons $m^{-2}s^{-1}$ and a temperature of 37±2^{o}C. Canonical correspondence analysis between obtained species and physico-chemical parameters of water revealed that temperature and HCO_3^- content were most influential parameters responsible for differential species distribution in summer and monsoon seasons respectively. High production of carotenoids in summer justified its role in protecting cellular constituents from photooxidation within microbial mats in response to higher light and temperature.

Introduction:

Cyanobacteria are the autotrophic oxygen evolving photosynthetic microscopic prokaryotic organisms responsible for the first oxygen built up in Earth's atmosphere (Schopf, 1993). Geothermal springs are one of the habitats where they grow profusely forming multilayered mat sturctures. The cyanobacterial diversity in most of the geothermal springs, distributed throughout the world, has been extensively documented (e.g. Castenholz, 1969, 1996; Brock, 1967, 1978; Ward *et al.,* 1987, 1989, 1998; Kastovsky and Komárek, 2001; Ward and Castenholz, 2002; Sompong *et al.*, 2005, Debnath *et al.* 2009; Roy *et al.,* 2014, 2015).The present work is part of a series of investigations undertaken on the cyanobacterial diversity and distribution of hot springs located in eastern India. Following the previous sequential contributions, on cyanobacterial flora of various geothermal springs of West Bengal (Debnath *et al.,* 2009; Roy *et al*., 2014, 2015 and Bhattacharya *et al.*, 2016), this article presents cyanobacterial diversity within mats collected from a previously unexplored hot spring located at Sidpur village, Pakur district, Jharkhand (N 24^{o}21'15.3", 87^{o}38'21.9") for the first time.

In India, cyanobacterial diversity of hot springs have been documented from western part of India (Thomas and Gonzalves, 1965; Vasistha, 1968), from Bihar (Jha, 1992; Jha and Kumar, 1990), from Orissa(Adhikary, 2006; Adhikary and Sahu, 1987), from Uttarakhand (Bhardwaj and Tiwari, 2010; Bhardwaj *et al.,* 2010) as well as From West Bengal (Debnath *et al.,* 2009; Roy *et al.,* 2014, 2015 and Bhattacharya *et al.,* 2016).However, there are unexplored areas and the hot water spring at Sidpur, Jharkhand is one such. The major aim of this study was to explore the diversity of cyanobacteria from the hot spring of this location to study morphometric details of collected species and to establish a correlation between their distribution and varying hydrological parameters in two different seasons.

Materials and Methods:

Collection site: The study area belongs to the Chhotanagpur plateau of Precambrian origin in the eastern part of India. This area is situated in Sidpur village (N 24^{o}21'15.3", E 87^{o}38'21.9") under Pakuria police station, Pakur district of Jharkhand State (Fig. 1). At the sampling site (approximately 1,200 sq. ft. in area) hot water emerges from underground source within a paddy field. Runoff water flows about 25 meters and gets stored in a shallow ditch. Formation of thin algal zone above sandy soil has been noticed in the hot spring water. The area surrounding the geothermal spring is having a faint smell of sulphur. Local people use this water body for bathing and various other purposes.

Fig. 1. Map location of Sidpur hot spring, Jharkhand

Sampling and morphometric analysis: Cyanobacterial mat samples were collected using 15cm x 15cm quadrats from hard and soft surfaces from the four sites in April (summer) and July (monsoon) in the year 2016. Mats were layered and each successive layer was peeled off, cyanobacterial species were removed by forceps and later observed by light microscopy for identification of the cyanobacterial species. Microscopic observations were undertaken within 24 hours of sample collection with temporary preparations made using 10% glycerine. Microphotographs were taken using a Leica trinocular DM 2500 microscope with a high power (40X) objective lens using bright field optics. Water samples were collected in sterile plastic bottles (1000ml) from each site for analysis of physico-chemical parameters.

Isolation from mat: The mat samples were washed repeatedly by double distilled water till the total removal of sand, clay, debris and particulate matters. Then the mat was homogenized by glass homogenizer and placed in Petri plates with agar medium as well as in conical flasks containing liquid BG-11 medium. All cultured samples were incubated at 37±2°C at a light intensity of 30-35 μmol photons m^{-2} s^{-1} under 40 W cool fluorescent tube on a 12 h:12 h light : dark cycle. Unicyanobacterial cultures were obtained under aseptic conditions by using streak-plate cultures on agarised (1.2 % w/v) BG-11 medium. Formed colonies consisting of a single species were then transferred to liquid culture medium.

Identification: Cyanobacterial species were identified following Anagnostidis and Komárek (1985, 1988, 1990), Komárek and Anagnostidis (1986, 1989) and Komárek (2003, 2013). Voucher specimens were kept in 4% formalin assigning respective cyanobacterial culture number in Visva-bharati Cyanobacterial Culture Collection (VBCCC).

Relative species abundance: Counts of individual species were made using a Sedgwick-Rafter chamber (Clescerl *et al.,* 1995) using the original mat samples. Relative abundance of each species was determined following Dash (2001).

$$\text{Relative abundance} = \frac{\text{Number of hits made on the species}}{\text{Total number of hits made}} \times 100$$

Physico-chemical analysis of spring water: Hot spring water temperature was measured by mercury thermometer in the field. pH values of the samples were determined by digital pH meter (Orion; Thermo Fisher Scientific, Waltham, USA) and electrical conductivity (EC) was measured using a conductivity bridge (Systronics, Norcross, USA). Redox potential was measured using Redox electrode (Orion; Thermo Fisher Scientific, USA). Sodium (Na^+), Potassium (K^+) and Calcium (Ca^{++}) concentrations were determined by flame photometer (Systronics). Ammonium (NH_4^+) was measured using an NH_4^+ electrode (Orion, Thermo Fisher Scientific, USA) attached to a multiparameter meter. Sulphate (SO_4^{-2}) was estimated following Chattopadhyay (1998). Dissolved oxygen (D.O.) and Chlorine content (Cl^-) were determined following Clescerl *et al.* (1995). Inorganic phosphate (PO_4^{-3}), Carbonate (CO_3^{-2}) and Bicarbonate (HCO_3^-) contents were estimated by titrimetric method following Trivedy and Goel (1984). Minimum detection limit (MDL) for E.C. was 0.001 mS cm^{-1},for PO_4^{-2} was 0.005 mg L^{-1};

for D.O., Cl^- and NH_4^+ was 0.01 mg L^{-1}; for Na^+, K^+, Ca^{++} and SO_4^{-2} was 0.1 mg L^{-1}; for CO_3^{-2} and HCO_3^- was 0.5 mg L^{-1}.

Canonical Correspondence Analysis: Canonical Correspondence Analysis (CANOCO version 4.5; ter Braak, 1986) was used to understand the correlation between physico-chemical parameters of hot spring water and cyanobacterial mat community in summer and monsoon seasons. Biplot scores of the environmental parameters were noted. CCA ordination was tested for significance with a Monte Carlo test (500 runs) using CANOCO software.

Pigment characterization: 90% methanol was used for extraction of chlorophyll-a and carotenoids from mat samples. For each mat community chlorophyll-a and carotenoid concentrations were measured and the carotenoid: chlorophyll-a ratio was determined using the method of Mackinney (1941). Homogenized cyanobacterial mass was centrifuged at 4000 rpm for 20 mins. The supernatant was discarded and 90% metanol was added to the pellet. Then tubes were kept at 4°C for 30 mins and then vortexed for 30 s and placed in boiling wáter bath at 60°C for 30 sec (avoiding the bubbling of extract). The supernatant was taken as extract of pigments. Absorbance value was measured at 663 and 750 nm by Spectrophotometer (Shimadzu UVPC-3101PC UV-VIS-NIR) to obtain Chlorophyll-a and carotenoids content. Subsequently, carotenoid: chlorophyll-a ratio was calculated. t-Test was performed to test the significance of the differences between the mean values for chlorophyll-a, carotenoids and carotenoids : chlorophyll-a ratio.

c-Phycocyanin and c-Allophycocyanin were extracted and quantified following Bennett and Bogorad (1973) using UV2700 Double beam UV-VIS Spectrophotometer (Chemito Spectrascan).

Results

Physico-chemical parameters of hot spring water:

Water temperature at the source of hot water has been found to vary between 54 and 56°C in both summer and winter seasons. Temperature gradually decreased from source and stood between 44 and 45°C in the runoff water. Water temperature recorded in the banks of the water body was between 42 and 43°C in both seasons. Chemical analysis of spring water showed that all the water samples exhibit high concentrations of Cl^- (79.52–103.66 mg L^{-1}) and high CO_3^{-2} (75–205 mg L^{-1}) content. Physico-chemical parameters studied in summer and monsoon seasons are given in Table 1.

Characterization of cyanobacterial mat communities

Detailed morphological and morphometric study of cyanobacterial species from the microbial mats and floating masses of four sampling spots of Sidpur hot water spring in two different seasons revealed the presence of 16 species of thermophilic and thermotolerant cyanobacteria spread over 11 genera (Table 2). Among these 11 genera 7 are non-heterocystous and 4 are heterocystous. The cyanobacteria isolated from mat samples belonged to all morphological categories of cyanobacteria - Unicellular (*Synechococcus, Thermosynechococcus, Chroococcus, Gloeocapsa*), non-heterocystous filamentous forms (*Oscillatoria, Leptolyngbya, Phormidium*), heterocystous, uniseriate, filamentous forms (*Desmonostoc, Nostoc*) and heterocystous, branched multiseriate filamentous forms (*Chlorogloeopsis, Mastigocladus*). Based on the dominant principal cyanobacterial component, the microbial mats were categorized into following mat types:

1. *Leptolyngbya* type: The uppermost green layers of multilayered cyanobacterial mats collected majorly from high temperature zones (S1 and S2; 54-56°C) were majorly composed of *L. fragilis* and *L. laminosa*. Multilayered mid-part of the mats were composed of species of *Leptolyngbya, Thermosynechococcus* and *Synechococcus* to form a complex mat structure in which filaments were embedded in thick gelatinous matrix.

2. *Phormidium* type: In some parts of middle layers (dull green in colour) abundance of *P. tenue,* and *P. amphibium* were observed in an intermingled fashion. These mats were observed majorly in lower temperature sites (S3 and S4; 42-48°C).

3. Thermophilic *Synechococcus* type: Apart from associated occurrence with species of *Leptolyngbya,* in some spots of high-temperature zones (54-56°C) of this geothermal area orange coloured, thin layer of cyanobacterial mats were observed that consisted of thermophilic *S. lividus, S. bigranulatus* and *T. elongatus.* Species of *Leptolyngbya* were also observed in these mats at lower frequency.

4. *Mastigocladus* type: Innermost layers of these mats (S2; 54°C) were dominated of *Mastigocladus laminosus.* Besides, species of *Leptolyngbya* and *Phormidium* were also observed in less frequency from these mats.

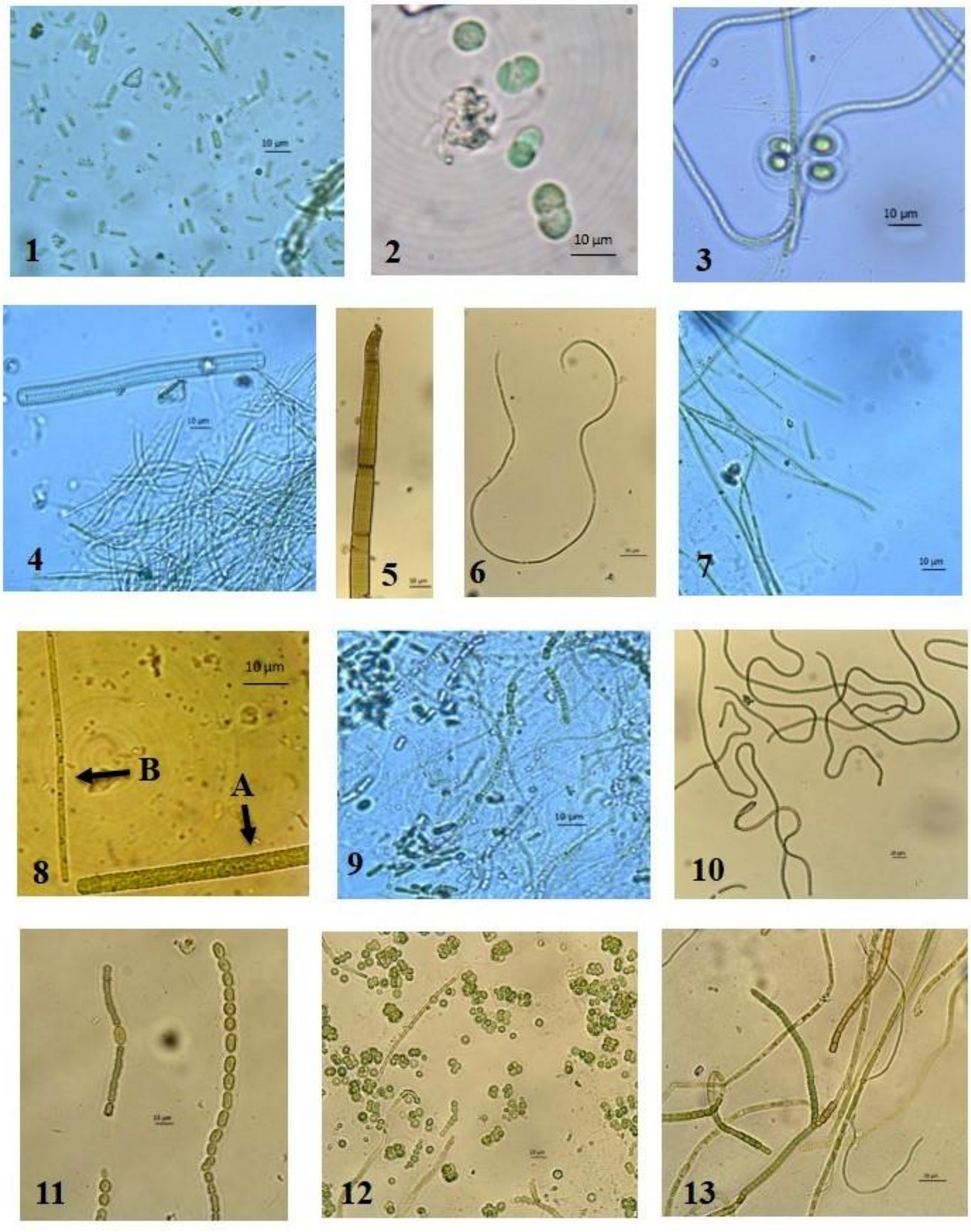

Plate 1. Microphotographs of collected cyanobacterial taxa from four sampling points of Sidpur hot water spring. Fig. 1. Population of *Thermosynechococcus elongatus* intermixed with Thermophilic *Synechococcus lividus* and *S. bigranulatus;* fig. 2. *Gloeocapsa gelatinosa;* fig. 3. *Chroococcus thermalis;* fig. 4. *Oscillatoria limosa* and *Leptolyngbya fragilis*; fig. 5. *O. princeps*; fig. 6. *Phormidium mucosum;* fig. 7. *Leptolyngbya laminosa;* fig. 8. A: *O. limosa*, B: *P. amphibium;* fig. 9. *Phormidium tenue;* fig. 10. *Desmonostoc muscorum;* fig. 11. *Nostoc paludosum;* fig. 12. *Chlorogloeopsis fritschii;* fig. 13. *Mastigocladus laminosus*.

Plate 2. Sampling site of Sidpur hot water spring. Fig. 1. Hot spring site encircled by two rounded concrete works; fig. 2. Reservoir of geothermal water that comes out of the spring source; fig. 3. Floating cyanobacterial mats; fig. 4. Drainage system containing run-off water.

Table 1: Results showing values of different physico-chemical parameters of Sidpur hot spring (S1 to S4: four sampling sites, ref. Plate 2; fig. 2)

Season	**Summer**				**Monsoon**			
Sites / Parameters	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
Temp (^{o}C)	56	54	44	42	55	54	48	42
pH	7.82	7.93	7.97	7.78	7.56	7.92	7.39	7.72
EC (mS cm^{-1})	0.710	0.746	0.748	0.785	0.656	0.671	0.729	0.75
Redox potential (mV)	63.7	48.1	46.3	67.9	83.6	53.5	88.1	68.1
DO (mg L^{-1})	6.689	6.486	6.689	7.094	7.297	7.018	7.297	7.283
Na^{+} (mg L^{-1})	27.5	28.7	22.8	23.5	28.6	30.9	25.1	27.5
K^{+} (mg L^{-1})	2.2	2.1	1.8	1.9	2.7	2.9	2.2	2.2
Ca^{++} (mg L^{-1})	15.8	16.8	18.8	17.5	7.6	9.1	8.7	8.7
Cl^{-} (mg L^{-1})	79.52	86.62	89.46	88.04	99.40	103.66	102.24	100.82
PO_4^{-2} (mg L^{-1})	0.07	0.12	0.18	0.19	0.225	0.215	0.230	0.223
SO_4^{-2} (mg L^{-1})	12.1	7.8	5.5	6.5	10.5	7.8	8.7	8.4
NH_4^{+} (mg L^{-1})	0.182	0.193	0.147	0.125	0.098	0.07	0.129	0.121
CO_3^{-2} (mg L^{-1})	80	75	95	92.5	185	180	202.5	205
HCO_3^{-} (mg L^{-1})	7.5	15	25	17.5	27.5	17.5	37.5	35

Table 2: Relative species abundance data obtained from mat samples of Sidpur hot spring (Plate 1; fig. 1-13)

Site	Species code in CCA	Summer				Monsoon			
		S1	S2	S3	S4	S1	S2	S3	S4
Temperature (°C)		56	54	44	42	55	54	48	42
Synechococcus lividus Copeland	*S. liv*	+++	++	-	-	++	++	-	-
Synechococcus bigranulatus Skuja	*S. big*	+++	+++	-	-	+++	++	+	-
Thermosynechococcus elongatus Katoh	*T. elo*	+++	++	-	-	+++	++	-	-
Gloeocapsa gelatinosa (Menegh.) Kützing	*G. gel*	+	++	+	++	+	+	++	+
Chroococcus thermalis (Menegh.) Nägeli	*Ch. the*	+	+	-	-	+	+	+	-
Oscillatoria princeps Vaucher ex Gomont	*O. pri*	-	-	++	++	-	-	+++	++
Oscillatoria limosa Agardh. ex Gomont	*O. lim*	-	-	+	+	-	-	+	+
Leptolyngbya fragilis (Gomont) Anagnostidis et Komárek	*L. fra*	++	+++	+	+	+++	++	++	+
L. laminosa (Gom.) Anagnostidis et Komárek	*L. lam*	+	++	++	+	+	++	++	++
Phormidium tenue (Menegh.) Gomont	*P. ten*	-	+	+	-	-	+	+	+++
P. mucosum Gardner	*P. muc*	-	+	+	+	-	+	+	+
P. amphibium (Agardh ex Gom.) Anagnostidis et Komárek	*P. amp*	-	-	++	++	-	-	++	++
Desmonostoc muscorum (Agardh ex Bornet et Flahault) Hrouzek et Ventura	*D. mus*	-	-	++	+	-	-	++	+
Nostoc paludosum (Kützing ex Bornet et Flahault)	*N. pal*	-	-	++	+	-	+	++	++
Chlorogloeopsis fritschii (Mitra) Mitra et Pandey	*Cl. fri*	-	++	+	+	+	+	+	++
Mastigocladus laminosus Cohn ex Kirchner	*M. lam*	+	++	++	-	+	+++	+	-
No. of species in each spring		8	11	12	10	9	12	14	11

Relative species abundance: +…1-5%; ++…5-20%; +++ …30-50%; ++++…….50-90%

From the morphometric studies it is evident that distribution of species is different in higher and lower temperature zones as well as species diversity is much higher in lower temperature zones (42-48^{o}C) than higher temperature zones (54-58^{o}C). Presence of thermotolerant species of *Oscillatoria, Phormidium, Nostoc* and *Desmonostoc* enriched the species distribution and diversity in lower temperature regime. Species of *Leptolyngbya* exhibited widespread occurrence along a thermal gradient of 56^{o}C to 42^{o}C.

Canonical correspondence analysis

CCA was done to understand the correlation between physico-chemical parameters of spring water and cyanobacterial mat community of four sampling points (Figure 1) in summer (Table 1) and monsoon seasons (Table 2) of 2016.

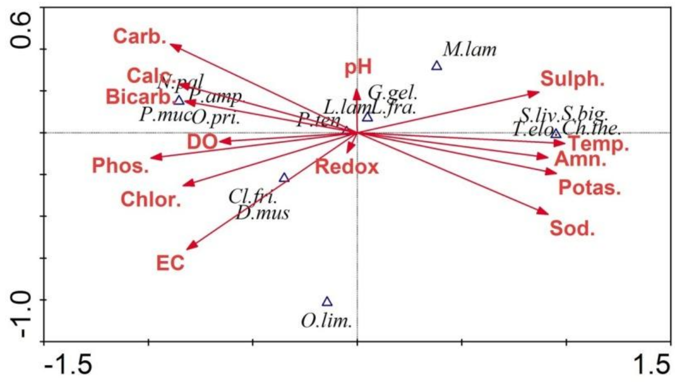

Fig. 2. CCA ordination biplot showing species-environment correlation between species occurrence and physico-chemical parameters of geothermal spring water in summer season.

a. **Summer**: The result from the ordination diagram of summer sampling (Fig. 2) indicates that the environmental parameters may be ranked in terms of their influence on species distribution considering the length of the arrows as follows - temperature, PO_4^{-2}, EC, Na^+, CO_3^{-2}, K^+, NH_4^+, SO_4^-, Ca^{++}, Cl^-, HCO_3^-, DO, pH and redox potential. The relative weight for the parameters indicates a wide range of variation (0.4296 - 0.0592) with a p-value of 0.1. The fraction of variance explained by corresponding axes (Axis 1: 0.435, Axis 2: 0.065, Axis 3: 0.059) indicates that Axis 1 is mostly contributing in explaining species – environment relationship. The Axis 1 explains the variance in excess of 77%. Axis 1 bears strong positive correlation with temperature (0.9929), K+ (0.9531), NH_4^+ (0.9119), Na^+ (0.9122), SO_4^- (0.8688), and negative correlation with PO_4^{-2} (-0.9877), CO_3^{-2} (-0.8950), Ca^{++} (-0.8554), Cl^- (-0.8337), HCO_3^- (-0.8264), EC (-0.8164) and DO (-0.6583). Axis 2 is negatively correlated with EC (-0.5594). The first three eigen values reported above are canonical; the fourth is not since only three independent constraints can be formed from the environmental variables. Thus plotting of the species in ordination diagram can be interpreted in terms of environmental gradient regarding first three axes. According to the ordination diagram (Fig. 2), *S. lividus, S. bigranulatus, T. elongatus* and *C. thermalis* occur at sites defined by high temperature, high concentrations of NH_4^+, K^+ and SO_4^-. *O. princeps, P. mucosum, P. amphibium* and *N. paludosum* occupy sites characterized by high concentration of HCO_3^- and Ca^{++}. *P. tenue* shows affinity towards sites having low pH whereas *G. gelatinosa, L. laminosa* and *L. fragilis* exhibit tendency towards sites defined by increasing pH. The occurrence of *D. muscorum* and *C. fritschii* is correlated with increasing electrical conductivity of water (Fig. 2).

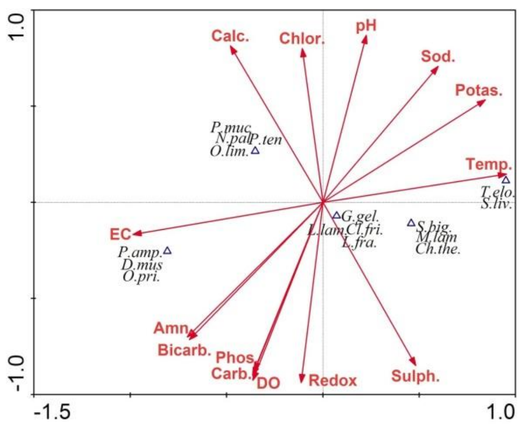

Fig. 3. CCA ordination biplot showing species-environment correlation between species occurrence and physico-chemical parameters of geothermal spring water in monsoon season.

b. Monsoon: From the CCA ordination diagram (Fig. 3) of monsoon season from Sidpur thermal spring, the environmental parameters may be ranked as per their influence on species distribution as follows - HCO_3^-, K^+, EC, NH_4^+, DO, Na^+, CO_3^{-2}, SO_4^-, temperature, PO_4^{-2}, Ca^{++}, redox potential, pH and Cl^-. The relative weight for the parameters indicates a narrow range of variation (0.2882–0.0376) with a p-value of 0.052. Here also the fraction of variance explained by corresponding axes (Axis 1: 0.295, Axis 2: 0.040, Axis 3: 0.025) indicate that the first axis is explaining the species – environment relationship. The first axis explains the variance of 82%. This axis bears positive correlation with temperature (0.9534), K^+ (0.8434) and Na^+ (0.5984) as well as negatively correlated with EC (-0.9858), NH_4^+ (-0.7034) and HCO_3^- (-0.6922). According to the ordination diagram (Fig. 3), *S. lividus* and *T. elongatus* show tendency towards sites characterized by higher temperature. *P. amphibium, O. princeps* and *D. muscorum* occur at sites with high electrical conductivity. Combined effect of temperature and SO_4^- has led to the distribution of *S. bigranulatus, C. fritschii* and *M. laminosus*. *P. tenue, P. mucosum, O. limosa* and *N. paludosum* exhibit affinity towards sites having increasing Ca^{++}. Species like *G. gelatinosa, L. fragilis, L. laminosa* and *C. fritschii* exhibit their distribution at sites having relatively low SO_4^- concentration as well low nutritive requirements as these species occupy at proximity of origin of the biplot as well as at the base of the arrow representing SO_4^-.

According the length of the arrow corresponding redox potential and occurrence of species in bi-plots, this parameter does not have much significance in species distribution in summer. Obtained values (46.3 – 88.1 mV) indicate oxidising condition of hot spring water**.**

Pigments analysis: Season wise variation in pigment composition i.e., chlorophylla-a (Chl-a), carotenoids and phycobiliproteins (C-PC and APC) of cyanobacterial mat samples collected from different sites (S1, S2, S3 and S4) of Sidpur hot water spring are summarized in table 3. In summer, carotenoid: chl-a ratio is higher as compared to those obtained in monsoon in all studied mat samples. Amongst the summer mat samples Site 2 (S2) exhibited highest value (0.678±0.045) of carotenoid: chl-a ratio. In monsoon, up to 0.186±0.009 of carotenoid: chl-a ratio has been observed. In summer season, S2 and S4 has exhibited high concentration of c-phycocyanin (79.79±3.63 µg/ml) and allophycocyanin (35.09±2.54 µg/ml) respectively as compared to those of other sites (Table 3).

Increase of carotenoids and carotenoids : Chl-a ratio in summer with respect to that of monsoon is significant at α <0.05.The decrease in chlorophyll-a in summer with respect to that of monsoon is not significant at α < 0.05. Increase of C-PC in summer with respect to that of monsoon is also significant at α <0.05.

Table: 3. Seasonal variation in carotenoids and chlorophyll-a ratio, C-Phycocyanin and Allophycocyanin content from cyanobacterial mat samples collected from four sites ofSidpur hot water spring

Sites	Summer			Monsoon		
	Carotenoid: Chl-a ratio	C-PC ($\mu g\ ml^{-1}$)	APC ($\mu g\ ml^{-1}$)	Carotenoid: Chl-a ratio	C-PC ($\mu g\ ml^{-1}$)	APC ($\mu g\ ml^{-1}$)
S1	0.416±0.037	50.76±1.79	5.18±0.47	0.136±0.009	20.72±0.89	1.13±0.12
S2	0.678±0.045	79.79±3.63	4.28±0.35	0.146±0.001	73.75±4.97	3.99±0.31
S3	0.34±0.018	29.27±1.86	-	0.186±0.009	16.83±1.21	-
S4	0.536±0.02	10.89±1.7	35.09±2.54	0.175±0.006	8.84±0.49	1.88±0.09

Discussion

This is the first systematic report on the distribution and diversity of mat forming thermophilic and thermotolerant cyanobacteria from Sidpur hot water spring in the district of Pakur, Jharkhand. The sampling site, like many other volcanic alkaline springs around the world (Ferris *et al.*, 1996; Sompong *et al.,* 2005; Debnath *et al.,* 2009; Ward and Castenholz, 2012; Arman *et al.,* 2014), exhibits high concentration of Cl^- (79.52–103.66 mg L^{-1}), CO_3^{-2} (75–205 mg L^{-1}) as well as pH up to 7.97. Observed temperature of water indicates the constancy of temperature throughout the year (Castenholz, 1969; Brock, 1978, Olivier, 2008). The distribution and composition of cyanobacterial species is quite similar to those reported by Debnath *et al.* (2009) and Roy *et al.* (2015) from Bakreswar geothermal province of West Bengal, by Roy *et al.* (2014) from Panifala thermal spring, Burdwan district, West Bengal and Bhattacharya *et al.* (2016) from Meteldanga and Khorasinpur in Birbhum district, West Bengal. This study reports the exclusive presence of *C. thermalis, O. limosa, P. mucosum, D. muscorum* and *N. paludosum* for the first time which have not been reported from geothermal springs of India so far. Formation of thick multilayered mats comprising differential species composition is a key feature of hot springs across the world (Castenholz, 1969; Brock, 1978; Sompong *et al.*, 2005; Klatt *et al.,* 2011). The present study agrees with the observation of Castenholz (1998, 2015) and Boomer *et al.* (2009) as multilayered mats have been abundantly observed especially in high temperature zones (54-56^{o}C). It has been observed, from statistical data, that temperature is one of the most influencing factors regarding the diversity and distribution of thermophilic and thermotolerant cyanobacterial species in geothermal springs (Brock, 1978; Roy *et al.*, 2015; Bhattacharya *et al.,* 2016). Occurrence of unicellular thermophilic *Synechococcus* in layered mats is universal in majority of geothermal springs at a temperature range of 50°C to 75°C (Ferris *et al.*, 1996; Ferris and Ward, 1997; Ward and Castenholz, 2002; Debnath *et al.*, 2009; Roy *et al.,* 2014, 2015; Bhattacharya *et al.,* 2016). Among the species isolated from the study site, thermophilic *S. lividus, S. bigranulatus* and *T. elongatus* have been isolated from the high temperature sites A and B (Plate 2, fig. 2), where the temperature ranges between 54°C and 56°C. Affinity of *S. lividus, S. bigranulatus* and *T. elongatus* towards high temperature at S1 (55-56^{o}C) and S2 (54^{o}C) in summer and monsoon seasons (Figs. 1 and 2) resembles the results of Roy *et al.* (2015). Presence of non-heterocystous filamentous species of *Leptolyngbya* at lower end of temperature gradient (42-48^{o}C) as well as their abundance in microbial mats at higher temperature (up to 56^{o}C) supports earlier findings (Johansen *et al*., 2008; Coman *et al*., 2013; Dadheech *et al*., 2013). Distribution of thermotolerant filamentous species like *P. mucosum, P. amphibium, O. princeps, D. muscorum* and *N. Paludosum* at lower temperature sites (S3 and S4) which is repesented in CCA biplot (figs. 2 and 3) is supported by the works of Sompong *et al.* (2005) and Debnath *et al.* (2009). Sompong *et al.* (2005) established a significantly negative correlation between cyanobacterial diversity and increasing temperature within geothermal environments. In the present study, decrease in cyanobacterial diversity with increasing temperature has been observed in S1 in both seasons. Increased species diversity in S3 as well as S2 in relatively low temperature regimes supports earlier observations of Castenholz (1969), Debnath *et al.* (2009) and Roy *et al.* (2015). Further, decrease in species abundance in S4 may be due to suboptimal condition with respect to geothermal habitats. 42^{o}C temperature of run-off water seems to act as stressor for most of thermophilic members (Ward and Castenholz, 2002). Yoneda (1952) documented the abundance of filamentous species of cyanobacteria from sites rich in CO_3^{-2} content. Similar observations are reported here as species of *Phormidium, Oscillatoria, Desmonostoc and Nostoc* have been obtained from S3 and S4 which are characterized by at high CO_3^{-2} concentration (up to 205 mg L^{-1}). Ward and Castenholz (2002), Sompong *et al.* (2005) and Debnath *et al.* (2009) concluded that NH_4^+ content has significant role in species distribution in cyanobacterial mat communities. Considering the fact that heterocystous cyanobacteria fix atmospheric nitrogen and leach ammonium to the substrata (Mager, 2009), heterocystous cyanobacteria *M. laminosus, C. fritschii, D. muscorum* and *N. paludosum* are found to be dominant in sites S2 and S3 where NH_4^+ content in water is high. Values of redox potential indicates oxidising condition of water as compared with that of hot springs of Taiwan where redox potential value ranged from -23 to -277 mV signifying reducing condition (Chen and Sung, 2008).

Increased production of carotenoids in response to higher light and temperature in summer is indicative of the facts that carotenoids protect cellular constituents and chlorophyll pigment (Krinsky, 1966; Castenholz, 1969). Our observation that high amount of carotenoids are produced at all four sites (S1, S2, S3 and S4) (Table 3) in summer season evidently supports the protective role of carotenoids to prevent photodesturction of chlorophyll-a in higher light and temperature.

Pumas *et al.* (2011) quantified phycobiliproteins content in two thermotolerant cyanobacteria –in *Leptolyngbya* sp. (KC45) up to 181.63 mg g^{-1} dry weight and rom *Phormidium* sp. (PD40-1) 165.47 mg g^{-1} dry weight. It is evident from bar diagram (Pumas *et al.,* 2011) that the c-PC values are nearly 42 and 60 mg g^{-1} dry weight respectively from *Leptolyngbya* sp. and *Phormidium* sp. In present work, higher value of c-PC up to 79.79±3.63 µg ml^{-1} was estimated in cyanobacterial mat dominated by *Synechococcus-Leptolyngbya-Mastigocladus* by freeze-thaw method (Table 3). Further detail study will help us understand the adaptive role of phycobiliproteins in hot water springs.

Bibliography

Adhikary, S.P., 2006. *Blue Green Algae: Survival Strategies in Diverse Environment*. Pointer Publication, Jaipur, pp. 156-172

Adhikary, S.P. and J. Sahu, 1987. Limnology of the thermal springs of Orissa. *J. Bombay Nat. Hist. Soc.* **84**: 497-502

Anagnostidis, K. and J. Komárek, 1985. Modern approach to the classification system of cyanophytes: 1-Introduction. *Algol. Stud*. **38/39**: 291-302

Anagnostidis, K. and J. Komárek, 1988. Modern approach to the classification system of cyanophytes: 3-Oscillatoriales. *Algol. Stud.* **50/53**: 327-472

Anagnostidis, K. and J. Komárek, 1990. Modern approach to the classification system of cyanophytes: 5-Stigonematales. *Algol. Stud.* **59**: 1-73

Arman, M., H. Riyahi, M. Yousefzadi and A. Sonboli, 2014. The study of cyanobacterial flora from Lamazan hot springs of West Hormozgan, Iran. *J. Life Sci. Biomed.* **4(3)**: 177-182

Bennett, A. and L. Bogoard, 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *The Journal of Cell Biology* **58**: 419-435

Bharadwaj, K.N. and S.C. Tiwari, 2010. Cyanobacterial diversity of two hyper-thermal spring, Ringidad and Soldhar in Tapoban geothermal field, Uttarkhand, Himalaya. *Curr. Sci.* **99:** 1513-1515

Bhardwaj, K.N., S.C. Tiwari and Y.M. Bahuguna, 2010. Screening of thermophilic cyanobacteria isolated from Tapoban geothermal field, Uttarakhand Himalaya for the production of antibacterial compounds. *Asian J. Exp. Biol. Sci.* **1(4)**: 787-791

Bhattacharya, S., S. Roy and S. Ray, 2016. Species composition of cyanobacterial component of mats collected from two hot springs of West Bengal, India – first report. *Phykos* **46 (1):** 32-39

Boomer, S.M., K.L. Noll, G.G. Geesey and B.E. Dutton, 2009. Formation of Multilayered Photosynthetic Biofilms in an Alkaline Thermal Spring in Yellowstone National Park, Wyoming. *Appl. Environ. Microbiol.* **75(8):** 2464-2475

Brock, T.D. 1967. Relationship between standing crop and primary productivity along a hot spring thermal gradient. *Ecology* **48**: 566-571

Brock, T.D., 1978. *Thermophilic microorganisms and life at high temperatures.* Springer-Verlag, Berlin, pp. 465

Castenholz, R.W., 1969. Thermophilic blue-green algae and the thermal environment. *Bacteriol. Rev.* **33**: 476-504

Castenholz, R.W., 1996. Endemism and biodiversity of thermophilic cyanobacteria. *Nova Hedwigia Beih.* **112**: 33-47

Chattopadhyay, G.N. 1998. *Chemical analysis of fish pond soil and water*. Daya publishing house, Delhi, pp. 132.

Chen, W.-F. and M. Sung, 2008. The redox potential of hot springs in Taiwan. *Terr. Atmos. Ocean. Sci.* **20(3):** 465-479

Clescerl, L.S., A.E. Greenberg and A.D. Eaton, 1995. *Standard methods for the examination of water and wastewater.* American Public Health Association, Washington D.C., pp. 1325

Coman, C., B. Drugă, A. Hegedus, C. Sicora and N. Dragos, 2013. Archaeal and bacterial diversity in two hot spring microbial mats from a geothermal region in Romania. *Extremophiles* **17:** 532-534

Dadheech, P.K., G. Glöckner, P. Casper, K. Kotut, C.J. Mazzoni, S. Mbedi and L. Krienitz, 2013. Cyanobacterial diversity in the hot spring, pelagic and benthic habitats of a tropical soda lake. *FEMS Microbiol. Ecol.* **85(2):** 389-401

Dash, M.C., 2001. *Fundamentals of Ecology*. Tata McGraw-Hill Publication, New Delhi, pp. 525

Debnath, M., N.C. Mandal and S. Ray, 2009. The Study of Cyanobacterial Flora from Geothermal Springs of Bakreswar, West Bengal, India. *Algae* **24(4):** 129-138

Ferris, M.J., A.L. Ruff-Roberts, E.D. Kopczynski, M.M. Bateson and D.M. Ward, 1996. Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. *Appl. Environ. Microbiol.* **62**: 1045-1050

Ferris, M.J. and D.M. Ward, 1997. Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* **63**: 1375-1381

Jha, M. and H.D. Kumar, 1990. Cyanobacterial flora and physicochemical properties of Saptadhara and Brahma Kund hot springs of Rajgir, Bihar, India. *Nova Hedwigia* **50**: 529-534

Jha, M., 1992. Hydrobiological studies on Suraj Kund and Chandrama Kund, hot springs of Rajgir, Bihar, India. *Int. Rev. Gesamten. Hydrobiol.* **77**: 435-443

Johansen, J.R., C.E. Olsen, R.L. Lowe, K. Fuckikova and D.A. Casamatta, 2008. *Leptolyngbya* species from selected seep walls in the Great Smoky Mountains national park. *Algol. Stud.* **12**: 21-36

Kastovsky, J. and J. Komárek, 2001. Phototrophic microvegetation of thermal springs in Karlovy Vary, Czech Republic. *Nova Hedwigia,* Beiheft **123:** 107-120

Klatt, C.G., J.M. Wood, D.B. Rusch, M.M. Bateson, N. Hamamura, J.F. Heidelberg, A.R. Grossman, D. Bhaya, F.M. Cohan, M. Kühl, D.A. Bryant and D.M. Ward, 2011. Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential, *The ISME Journal* **5:** 1262–1278

Komárek, J. 2013. Cyanoprokaryota, Part 3: Heterocytous Genera. In Büdel, B., Gärtner, G., Krienitz, L. and Schagerl, M. (eds.), *Süßwasserflora von Mitteleuropa,* Springer-Verlag, Berlin, Heidelberg: pp. 1130

Komárek, J. 2003. Problem of the taxonomic category "species" in cyanobacteria. *Algol. Stud.* **109**: 281–297

Komárek, J. and K. Anagnostidis, 1989. Modern approach to the classification system of cyanophytes 4 – Nostocales. *Algol. Stud*. **56:** 247–345

Komárek, J. and K. Anagnostidis, 1986. Modern approach to the classification system of cyanophytes 2 – Chroococcales. *Algol. Stud*. **43:** 157–226

Krinsky, N.I. 1966. The role of carotenoid pigments as protective agents against photosensitized oxidations in chloroplasts. In: *Biochemistry of chloroplasts.* Ed. Goodwin, T.W. Vol. 1, Academic Press Inc., New York, pp. 423-430

Mackinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. **140**: 315-322

Mager, D. M. 2009. Extracellular polysaccharides from cyanobacterial soil crusts and their role in dryland surface processes. Manchester Metropolitan University, Manchester, UK, pp. 273.

Olivier, J., H.J. van Niekerk and I.J. van der Walt, 2007. Physical and chemical characteristics of thermal springs in the Waterberg area in Limpopo Province, South Africa. *Water SA (on-line)* **34(2):**163-174

Pumas, C., P. Vacharapiyasophon, Y. Peerapornpisal, P. Leelapornpisid, W. Boonchum, M. Ishiiand C.Khanongnuch, 2012. Purification and Characterization of a Thermostable Phycoerythrin from Hot Spring Cyanobacterium *Leptolyngbya* sp. KC45. *Int. J. Agric. Biol*. **14:** 121–125

Roy, S., S. Bhattacharya, M. Debnath and S. Ray, 2015. Diversity of cyanobacterial flora of Bakreswar geothermal spring, West Bengal, India-II. *Algol. Stud.* **147**: 29-44

Roy, S., M. Debnath and S. Ray, 2014. Cyanobacterial flora of the geothermal spring at Panifala, West Bengal, India. *Phykos* **44 (1)**: 1-8

Schopf, J.W., 1993. Microfossils of the early Archean Apex Chert: new evidence of the antiquity of life. *Science,* **260(5108):** 640-646.

Sompong, U., P.R. Hawkins, C. Besley and Y. Peerapornpisal, 2005. The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand. *FEMS Microbiol. Ecol.* **52**: 365-376

Ter Braak, C.J.F. 1986. Canonical correspondence analysis: A new eigen vector technique for multivariate direct gradient analysis. *Ecology*, **67:** 1167–1179

Thomas, J. and E.A. Gonzalves, 1965. Thermal algae of Western India: VII. Algae of the hot springs at Rajapur. *Hydrobiologia* **26**: 66-71

Trivedy, R.K. and P.K. Goel, 1984. *Chemical and Biological Methods for Water Pollution Studies*. Environmental Publications, Karad, pp. 46-48

Vasistha, P.C., 1968. Thermal cyanophyceae of India – 1. *Phykos* **7**: 198-241

Ward, D.M., T.A. Tayne, K.L. Anderson and M.M. Bateson, 1987. Community structure and interactions among community members in hot spring cyanobacterial mats. *Symp. Soc. Gen. Microbiol.* **41**:179-210

Ward, D. M., Weller, R., Shiea, J., Castenholz, R.W. and Cohen, Y. 1989. Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Cohen Y. and Rosenberg E. (eds), *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*, American Society for Microbiology, Washington D.C., pp. 3-15.

Ward, D.M., M.J. Ferris, S.C. Nold and M.M. Bateson, 1998. A natural view of microbial biodiversity within hot spring mat communities. *Microbiol. Mol. Biol. Rev*. **62**: 1353-1370

Ward, D.M. and R.W. Castenholz, 2002. Cyanobacteria in geothermal habitats. In: *The Ecology of Cyanobacteria* Ed. Whitton, B.A. and M. Potts, Kluwer Acdemic Publishers, Dordrecht, pp 37-59

Yoneda, Y. 1952. A genereal consideration of the thermal Cyanophyceae of Japan. *Mem. Coll. Agr. Kyoto. Univ. Fish. Ser.* **62**:1-20