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Species composition of cyanobacterial component of mats collected from two hot springs of West Bengal, India – first report

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Abstract

This is the first report of species diversity of cyanobacterial flora present in the microbial mats of two slightly alkaline geothermal springs located at Meteldanga and Khorasinpur in Birbhum district of West Bengal. Five species of cyanobacteria found in the hot-spring at Meteldanga (49ºC) are –*Leptolyngbya laminosa*, *Phormidium tenue*, *Thermosynechococcus elongatus*, *Leibleinia gracilis* and *Phormidium amphibium*. Species identified from the hot spring at Khorasinpur (59ºC) are *Leptolyngbya laminosa*, *Phormidium breve*, *Aulosira bombayensis* and *Scytonema varium*. Of these *Leibleinia gracilis, Lyngbya hieronymusii* and *Aulosira bombayensis* are reported for the first time from the hot springs of West Bengal. Water of these two hot-springs was physico-chemically analysed with regard to nine parameters – pH, temperature, redox, Electrical conductivity, acidity, alkalinity, free CO_2, Dissolved oxygen, ammonium. On the basis of predominance of cyanobacterial species the mats were designated as *Synechococcus* type and *Leptolyngbya* type. *Synechococcus* type mat was found in Meteldanga and *Leptolyngbya* type mats were collected from both Meteldanga and Khorasinpur. *Leptolyngbya laminosa* was most abundant species in both geothermal springs. It was found to occurr with more diverse filamentous cyanobacteria in low temperature regions of hot springs. Correlation between water parameters and occurrence of cyanobacterial species in mats was analysed using CCA for both geothermal springs.

Key words – Cyanobacteria, hot spring, mat, species composition, West Bengal

Introduction

The present work is part of a series of investigations undertaken on the cyanobacterial flora of hot springs located in West Bengal. On previous occasion cyanobacterial flora were studied from hot springs located at Bakreswar and Panifala (Debnath *et al.*, 2009; Roy *et al*., 2014 and Roy *et al.*, 2015). In this article we are presenting for the first time cyanobacterial species composition of mats collected from two new hot springs located at Meteldanga and Khorasinpur in the district of Birbhum. Previously, cyanobacterial population from hot springs have been reported from states like Himachal Pradesh, Haryana, Maharastra, Gujrat, Bihar and Orissa (Vasistha, 1968; Thomas and Gonzalves, 1965; Jha, 1992; Jha and Kumar, 1990; Adhikary, 2006; Adhikary and Sahu, 1987; Bharadwaj and Tiwari, 2010; Bharadwaj *et al.*, 2010; Dash *et al.*, 2012 and Mongra, 2012). Jana (1973) while working on spring water potability and planktons from Bakreswar, worked on diversity of cyanobacterial flora of Bakreswar geothermal spring of West Bengal, Debnath *et al.* (2009) and Roy *et al.* (2014) and Roy *et al*. (2015) conducted through systematic study of the cyanobacterial flora from various geothermal springs of West Bengal. These studies involve study of distribution and detailed description of cyanobacterial species diversity of these geothermal springs along with physico-chemical parameters of the hot spring water. It is to be noted that each thermal spring is more or less constant in its physico-chemical features and has its own characteristic cyanobacterial population found throughout the year (Castenholz, 1969). There exists a distinct relation between the physico-chemical parameters of the water and cyanobacterial species distribution in a hot spring. So, in the present study, physico-chemical parameters of these two hot spring water have been worked out and these parameters have been correlated with species distribution of cyanobacteria.

Materials and Methods

Sampling sites

One of the two present sampling sites is a solitary thermal spring at Meteldanga (N 18°00 E 077°20.625') in the district of Birbhum, West Bengal. Hot water comes out from an artificially made concrete structure called the mouth and runs over the concrete surface.

The second sampling site is Khorasinpur (N 24°04.315' E 087°44.732') in the same district. Here hot water comes out from the mouth of an iron pipe which has been inserted at the source of the hot water.

Sampling and morphological study

Mat samples of cyanobacteria, formed on the surface near the mouth of the thermal spring, were collected using 15cmx15cm quadrate. Microscopic observations were undertaken within 24 hours of sampling to study the morphology of organisms forming the mats by making temporary slides using 10% glycerin. Microphotographs were taken using Leica trinocular DM 2500 microscope with photographic attachment. Identification of

cyanobacterial samples was done following Desikachary (1959) and Anagnostidis and Komárek (1985, 1988, 1990).

Relative species abundance

It was determined from cyanobacterial mat samples collected in quadrates from the sampling sites following Dash (2001) to obtain the quantitative value of species occurrence.

Physico-chemical analysis of spring water

Temperature: Spring water temperature was measured by thermometer in the field.

pH: pH values of samples were determined by Orion digital pH meter (Orion; Thermo Fisher Scientific, USA).

Acidity, Alkalinity and Free CO_2: Acidity, Alkalinity and Free CO_2 of spring water were estimated by titrimetric method (Trivedy and Goel, 1984).

D.O. : D.O. of spring water was estimated by Winkler's method.

Statistical analysis

Canonical Correspondence Analysis: CANOCO version 4.5 (Ter Braak, 1986) was used.

Results

Cyanobacterial species composition of the mats and samples collected from hot springs

Five cyanobacterial species were identified from mats collected from thermal spring located at Meteldanga. Four cyanobacterial species were identified from mats collected from the mouth of Khorasinpur hot spring. Here, three cyanobacterial species were intermingled sparsely with *Leptolyngbya laminosa*. One organism was collected from samples collected from bathing pond attached to the thermal spring (Table 1; Plate 1& 2).

More diversity was found in mats collected from Meteldanga than Khorasinpur. The species composition of upper and lower surface of the mat collected from the mouth of Meteldanga thermal spring differed. Mats collected from the place little away from the mouth contain cyanobacterial species other than those present in mats collected from the mouth (Table 1).

Table 1: Table showing the sampling sites and list of isolated cyanobacteria from mats

Meteldanga			**Khorasinpur**	
Upper surface of mat near mouth	Lower surface of mat near mouth	Mat from site away from mouth	Mat near mouth	Bathing pond
Leptolyngbya laminosa	*Phormidiun tenue* *Leptolyngbya laminosa*	*Thermosynecococcus elongatus* *Leibleinia gracilis* *Phormidium amphibium*	*Phormidium breve* *Leptolyngbya laminosa* *Lyngbya hieronymusii* *Aulosira bombayensis*	*Scytonema varium*

Plate 1: Sampling sites and collected cyanobacterial mats. Fig. 1. Sampling site of Meteldanga; Fig. 2. Sampling site of Khorasinpur; Fig.3.Upper surface of mat from mouth of Meteldanga; Fig. 4. Lower surface of mat from mouth of Meteldanga; Fig. 5. Mat from site away from mouth of Meteldanga; Fig. 6 & Fig. 7. Mat from Khorasinpur.

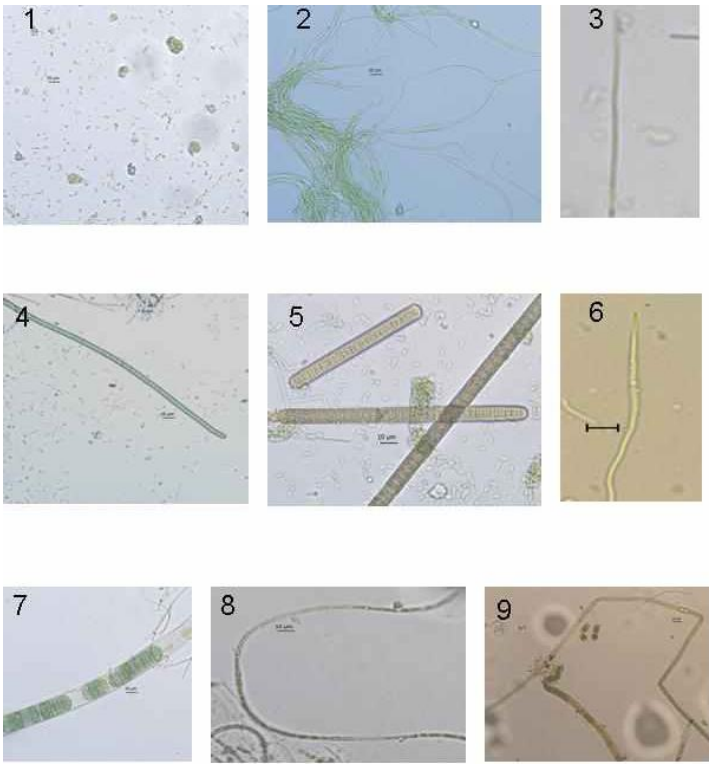

Plate 2: Microphotographs of cyanobacteria isolated from mats collected from Meteldanga and Khorasinpur. Fig.1. *Thermosynechococcus elongatus*; Fig. 2. *Leptolyngbya laminosa*; Fig. 3. *Phormidium amphibium*; Fig. 4. *P. Breve*; Fig. 5. *Leibleinia gracilis*; Fig. 6. *P. tenue*; Fig. 7. *Lyngbya hieronymusii;* Fig. 8. *Aulosira bombayensis*; Fig. 9. *Scytonema varium*

From morphometric exploration we observed that all the microbial mats in Meteldanga and Khorasinpur thermal springs are principally dominated by *Thermosynechococcus elongatus* and *Leptolyngya laminosa* are belong to i) Thermophilic *Synechococcus* type and ii) *Leptolyngbya* type respectively.

i) Thermophilic *Synechococcus* type: This mat type was dominated by *Thermosynechococcus elongatus*. Along with this species *Leibleinia gracilis* and *Phormidium amphibium* were present in lower frequency. This mat was found at distant position from the mouth of the thermal spring of Meteldanga.

ii) *Leptolyngbya* type: This mat type was dominated by *Leptolyngbya laminosa*. *Phormidium tenue* was present at lower frequency at the lower surface of the mat collected from the mouth of thermal spring of Meteldanga.

Phormidium breve, *Lyngbya hieronymusii* and *Aulosira bombayensis* were present in very low frequency at different layer of the mat collected from the mouth of the thermal spring of Khorasinpur. In both cases, the mats are characterized by thick gelatinous matrix within which the filaments are embedded.

Leptolyngbya laminosa was most abundant cyanobacterial species found in mats collected from both study sites (Table 2).

Table 2: Relative abundance of cyanobacterial species identified from mats collected from Meteldanga and Khorasinpur hot springs

Sl. No.	**Cyanobacterial taxa**	**Relative abundance in Meteldanga thermal spring**	**Relative abundance in Khorasinpur thermal spring**
1.	*Leptolyngbya laminosa* (Gomont) Anagnostidis et Komárek	++++	++++
2.	*Thermosynechococcus elongatus* Katoh	++++	-
3.	*Phormidium tenue*	+++	-
4.	*Phormidium amphibium*	+	-
5.	*Leibleinia gracilis* (Rabenhorst et Gomont) Anagnostidis and Komárek	+	-
6.	*Phormidium breve* (Kütz. ex Gom.) Anagnostidis et Komárek	-	+
7.	*Lyngbya hieronymusii* Lemm.	-	+
8.	*Aulosira bombayensis* Gonzalves	-	+

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

Canonical Correspondence Analysis (CCA)

CCA was done to understand the correlation between physico-chemical parameters of spring water and cyanobacterial mat community of both study sites (Table 3; Table 1). The result from the ordination diagram (Plate 3) indicates that the environmental parameters may be ranked in terms of their influence on species distribution considering the length of arrows – EC, D.O., ammonium, acidity, alkalinity, redox potential, temperature, free CO_2 and pH. The relative weight as denoted by extra-fit values for the parameters indicates a narrow range of variation (0.3480 - 0.4959) with a p-value of 0.0820. The fraction of variance explained by corresponding axes indicates that the first axis is mostly contributing in explaining species – environment relationship. Axis 1 bears most strong positive correlation with free CO_2 and most strong negative correlation with alkalinity where as axis 2 has most strong positive correlation with acidity and most strong negative correlation with pH.

Table 3: Table showing water parameters of thermal springs

Water parameters	Meteldanga	Khorasinpur
pH	6.8	7.5
Acidity (mg L^{-1})	3.9	2.5
Free CO_2 (mg L^{-1})	1.0	0.5
Alkalinity (mg L^{-1})	6.6	7.9
D.O. (mg L^{-1})	5.6	3.1
Redox (mV)	94.8	90.3
Temperature (ºC)	49	59
Ammonium (mg L^{-1})	0.872	0.373
EC (mS cm^{-1})	0.83	0.63

All four eigenvalues are canonical and correspond to axes that are constrained by the environmental variables.

According to the ordination diagram (Plate 3) filamentous cyanobacteria *Phormidium amphibium*, *Leibleinia gracilis* and unicellular cyanobacteria *Thermosynechococcus elongatus* occur at the base of the arrow representing temperature showing affinity towards sites with low temperature. Their occurrence is also influenced by low pH. *Leptolyngbya laminosa* shows affinity towards site with moderate temperature, alkalinity and pH. Occurrence of *Phormidium breve* and *Aulosira bombayensis* are influenced by temperature and pH. *Scytonema varium*, a heterocystous cyanobacteruium shows affinity towards increasing amount of ammonium as well as D.O., E.C., redox and acidity. *Phormidium tenue* also occurs with increasing D.O., E.C., redox and acidity.

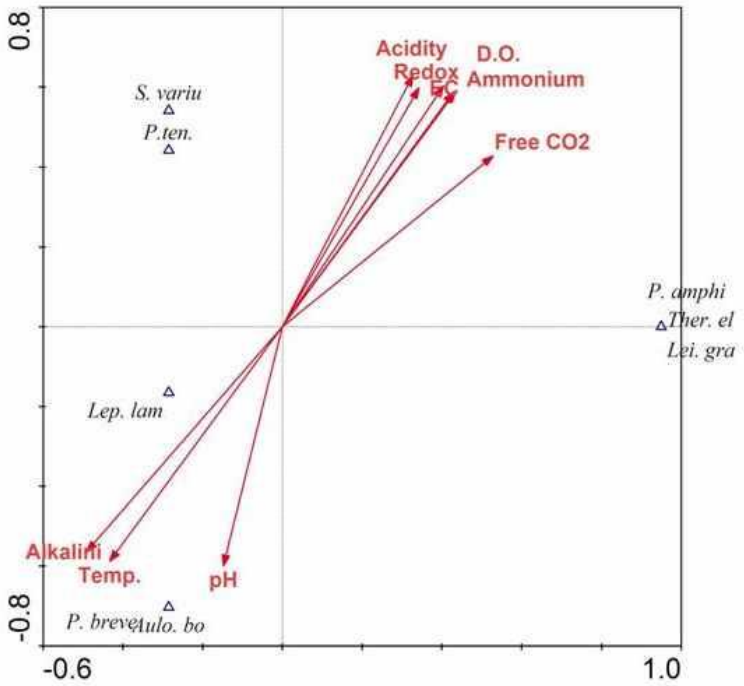

Plate 3: CCA ordination diagram showing correlation between cyanobacterial species and water parameters of Meteldanga and Khorasinpur thermal spring.

Discussion

This is the first sutdy of cyanobacterial species composition of mats collected from hotsprings located at Meteldanga and Khorasinpur situated in the district of Birbhum, West Bengal. Except three cyanobacterial species – *Leibleinia gracilis*, *Lyngbya hieronymusii* and *Aulosira bombayensis* all the cyanobacterial species are similar to those reported by Debnath *et al.* (2009), Roy *et al.* (2015) from different sites of Bakreswar thermal spring complex (district Birbhum) and by Roy *et al.* (2014) from Panifala thermal spring (district Bardwan) from West Bengal.

In geothermal springs temperature is one of the most important factors which influences the distribution and diversity of cyanobacterial species (Roy *et al.*, 2014). At 50ºC layered mats are formed by unicellular cyanobacterium – thermophilic *Synechococcus* (Ferris and Ward, 1997; Ferris *et al.,* 1996; Ward and Castenholz, 2002; Debnath *et al.*, 2009). Roy *et al.* (2014) and Roy *et al.* (2015) reported presence of *Thermosynechococcus* at a temperature of 54-61ºC. Occurrence of *Synechococcus* sp. along with other filamentous species like *Phormidium, Pseudoanabaena* and *Spirulina* was reported by Norris *et al.* (2002) from Yellow stone National park at a lower temperature of 40-47ºC. However the occurrence of this combination of species was more diverse than those present at higher temperature (60-80ºC). Newly studied locations, visited in the present study, also have occurrence of *Thermosynechococcus elongatus* at lower temperature along with filamentous cyanobacteria. The occurrence of *Leptolyngbya* is very frequent as reported by microscopy and sequencing (Albertano and Korácik, 1994; Ishida *et al.*, 2001; Casamatta *et al.*, 2005; Komárek and Anatgnostidis, 2005: Taton *et al*., 2006; Johansen *et al*., 2008; Bohunická *et al*., 2011). In Lake Bogoria, Kenya (40-80ºC) the occurrence of *Leptolyngbya* was most prevalent (Dadheech *et al*., 2013). Amarouche *et al.* (2014) reported morphological and phylogenetic diversity of thermophilic cyanobacteria in Algerian hot springs. The hottest springs were dominated by members of *Leptolyngbya*, *Synechococcus*-like cyanobacteria where as members of Oscillatoriales other than *Leptolyngbya*, Chroococcales and Stigonematales dominated lower temperature springs. In our observation *Leptolyngbya* have been found in both lower (49ºC) and higher temperature (59ºC). This indicates the adaptability of this genus at wider temperature range. At 55ºC, *Leptolyngbya* is one of the dominant cyanobacteria. Coman *et al*. (2013) reported the occurrence of *Leptolyngbya* as a dominant cyanobacterium from two slightly alkaline hot springs at 55ºc from the western plain of Romania. Occurrence of *Leptolyngbya* in the two hot springs under study (49 - 59ºC) corroborates earlier findings.

Ward and Castenholz (2002) and Sompong *et al.* (2005) emphasized the role of pH in species distribution. *Leptolyngbya laminosa* was found in slightly alkaline pH at Vairabkunda in Bakreswar geothermal spring (Roy *et al*., 2015). In present study, occurrence of *L. laminosa* at pH 6.8-7.5 supports the previous reports. CCA of present study also supports this view.

Following CCA ordination diagram, Debnath *et al.* (2009) reported that occurrence of *Phormidium breve* is influenced by temperature. In CCA ordination diagram of summer and monsoon of Bakreswar thermal spring, presence of filamentous forms in the lower temperature zone is reflected (Roy *et al*., 2015). CCA ordination diagram of present study supports the previous view. Effect of pH on occurrence of *Phormidium amphibium* was reported in CCA ordination diagram (Roy *et al*., 2015).Findings of the present study is similar to the previous reports.

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