Cyanobacterial flora of the geothermal spring at Panifala, West Bengal, India

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Abstract

This is the first report of diversity of cyanobacterial flora present in the microbial mats of an alkaline geothermal spring located at Panifala in Burdwan district of West Bengal. Ten species of cyanobacteria belonging to three heterocystous and four non-heterocystous genera from four cyanobacterial orders were isolated and identified by morphological and morphometric studies. The temperature range in the hot spring is 50-61°C. Temperature has been found to be the key factor for the variation of species composition in this spring. In addition to temperature, pH and electrical conductivity were also measured. Various chemical parameters like dissolved oxygen, phosphate, ammonium, bicarbonate, chloride and metals like sodium, potassium and calcium of the hot spring water were measured. An isolated strain of *Fischerella thermalis* was physiologically characterized with regard to pigment profile. In summer season carotenoid : chl-a ratio is 2.637:1 (highest among 5 sites) while in monsoon carotenoid : chl-a ratio is 0.132:1 (lowest among 5 sites). Species of *Synechococcus* and *Calothrix* were dominant in this solitary hot spring. The most abundant species were *Synechococcus bigranulatus*, *S. elongatus* and *Phormidium laminosum*. The genera *Synechococcus*, *Phormidium*, *Calothrix* and *Fischerella* were isolated from the above mentioned spring using BG-11 medium under 25-30 μmol photons $m^{-2}s^{-1}$ light and 37±2ºC temperature.

Keywords: Cyanobacteria, geothermal spring, species diversity

Introduction

Cyanobacteria originated in the early Precambrian (3.5 Ga) as the first oxygen evolving photosynthetic organisms. The environmental conditions during that period were defined by high temperature, anaerobic condition, high concentration of sulphur and reducing gases like methane, ammonia, CO_2 in the atmosphere. The ecological condition in geothermal springs is very similar to this and cyanobacteria have established themselves in this ecological habitat as a successful community. The cyanobacterial diversity in geothermal environment has been studied by many workers throughout the world (Castenholz, 1969, 1996; Brock, 1978; Ward *et al.*, 1987, 1998; Ward and Castenholz, 2002; Sompong *et al.*, 2005; Bhardwaj *et al.*, 2010). In India, few works have been done from western part by Thomas and Gonzalves (1965) and from Bihar and Orissa by Jha and Kumar (1990), Jha (1992) and Adhikary (2006). In West Bengal Debnath *et al.* (2009) documented the diversity of cyanobacterial flora of 4 thermal springs of Bakreswar geothermal spring complex and reported 18 species, distributed in 12 genera. Thermophiles are valued sources of thermostable biocompounds. Isolation and characterization of thermophilic cyanobacterial strains provides a good stock of organisms for future biotechnological exploration. Thermophilic cyanobacteria of geothermal springs in India in general and those of West Bengal in specific have not received much attention. Our present survey is the first systematic study of distribution and detailed description of cyanobacterial diversity of this particular geothermal spring located in Panifala, the district of Burdwan (23^{o}45'N, 86^{o}58'E), West Bengal.

Material and Methods

Sampling site

The present sampling site is a solitary thermal spring at Panifala (23 ^{o}45'33"N, 86 ^{o}58'54"E) in the district of Burdwan of West Bengal, India. Hot water comes out from an artificially made concrete structure called the 'mouth' and runs through the concrete drain (Fig. 1 in Plate 1).

Sampling and morphological study

Mat samples of cyanobacteria, formed on surface made up of ceramic tiles and the concrete drain carrying hot water were collected using 15cm X 15cm quadrates. Microscopic observations were undertaken within 24 hours of sample collection to study the morphology of organisms forming the mats by making temporary slides using 10% glycerine.

Morphological variations were also studied from culture plates where the mats were placed in agar medium. Microphotographs were taken using Olympus CH40 and Leica trinocular DM 2500 microscope with photographic attachment.

Isolation and identification

The mat samples were washed repeatedly by double distilled water, homogenized and placed in Petri plates and conical flasks containing BG-11 medium (both –N and +N). All cultured samples were incubated at 37±2°C under 25-30 μ mol photons m^{-2} s^{-1} light (12 h:12 h Light and dark cycle) in seed germinator to put the experimental materials under controlled condition. Unicyanobacterial cultures were obtained under aseptic condition by streaking the cells repeatedly on agar plate containing BG-11 medium and thereafter transferring those strains to liquid culture medium in conical flasks. Identification of cyanobacterial samples was done following Geitler (1932), Desikachary (1959), Rippka *et al.* (1979) and Anagnostides & Komarek (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

Spring water temperature was measured by thermometer in the field. pH values of the samples were determined by Orion digital pH meter (Orion; Thermo Fisher Scientific, USA) and electrical conductivity (EC) was measured using a Systronics conductivity bridge (Systronics, USA). Potassium (K^+), Calcium (Ca^{++}), Sodium (Na^+) contents were determined by Systronics flame photometer. Ammoniunm (NH_4^+) concentration was measured using Orion multiparameter meter NH_4^+ electrode; Chlorine content (Cl^-), dissolved oxygen (DO) and inorganic phosphate (PO_4^{-3}) content of water samples were determined following Clescerl *et al*. (1995). Bicarbonate (HCO_3) contents were measured following usual titrimetric method given by Trivedy and Goel (1984).

Pigment characterization

90% methanol was used for extraction of pigments (Chlorophyll-a and carotenoids) from each mat community from which chlorophyll-a contents, carotenoid contents and carotenoid : chlorophyll-a ratio was determined following the extraction and estimation method of Mackinney *et al*. (1941) using extinction coefficient.

Results

Species composition of the mats collected from hot springs

The identification of the cyanobacterial strains from the mat communities of the above mentioned alkaline thermal spring revealed the presence of 10 species spread over 7 genera which have been enlisted in the Table-1.

Table 1: List of identified cyanobacterial taxa and relative species abundance in Panifala in two smpling seasons (A to E: sampling sites, ref. Plate 1; Fig. 1)

Cyanobacterial taxa	**Relative abundance (Monsoon)**					**Relative abundance (Summer)**				
	A	B	C	D	E	A	B	C	D	E
Synechococcus bigranulatus Skuja		++	+++	+			+++	+++	+	
Synechococcus elongatus Nägeli		++	++	++			+++	+++	+	
Chroococcus minutus Näg.		+	+	+	+		+	+	+	+

Gloeocapsa sp. Kütz.	+	+	+	+		+	+	+	+	
Phormidium laminosum Gomont	+++	+	+	++	++	++	+++	+	++	+
Phormidium tenue (Menegh.) Gomont	+++			+++	++	++			+++	+++
Calothrix braunii Bornet et Flahault		++	+	+	+		++		+	+
Calothrix thermalis (Schwabe) Hansg.	+	++	++	+		+	++	+	+	
Scytonema millei Bornet ex Born. et Flah.						+			+	+
Fischerella thermalis (Schwabe) Gomont	+	+		++	++	+	+		+	++

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

From morphometric exploration we observed that all the microbial mats in Panifala are principally dominated by Chroococcalean and Oscillatorialean cyanobacterial members and belong to i) *Synechococcus* type and ii) *Phormidium* type according to Yoneda (1952).

1. *Synechococcus* type: *Synechococcus bigranulatus* and *Synechococcus elongatus* are two most abundant species in this geothermal spring surviving at higher temperature optima of 54-61°C. Variously coloured cyanobacterial mats dominated by species of *Synechococcus* firmly anchor to the solid undersurface made up of ceramic tiles. Presence of *Gloeocapsa* sp. and *Phormidium* sp. in an intermingled fashion with *Synechococcus* sp. is also noted here.

2. *Phormidium* type: This mat type has been observed in relatively lower temperature range (50-56°C) of Panifala hot spring. Cyanobacterial mats are formed at the sides of the water body having water flown from the source. The top layer of such mats consists of light green or blue-green filamentous *Phormidium laminosum* (most abundant) and *Gloeocapsa* sp. embedded in gelatinous matrix. The layer beneath this is composed of species of *Synechococcus*, *Phormidium laminosum*, *P. tenue* and *Gloeocapsa* sp. The only genus present in the run-off water is *Phormidium* sp. *Fischerella thermalis* was established as a pure culture after being isolated from mat samples in BG-11 medium by repeated culturing.

Physico-chemical parameters of hot spring water

Chemical analysis of spring water evidently shows that the thermal spring under survey exhibits higher level of Na^+ (78.5-121.5 mg L^{-1}) and Cl^- (149.41-155.33 mg L^{-1}) content along with high amount of bicarbonates (525-1360 mg L^{-1}). This indicates that Panifala hot spring belongs to volcanic Na, Cl-HCO_3 type of hot springs. This categorization of hot springs has been done following Castenholz (1969). Physico-chemical parameters studied in two different seasons (monsoon and summer) are given in Table-2.

Table 2: Results showing values of different physico-chemical parameters of Panifala hot spring (A to E: sampling sites, ref. Plate 1; fig. 1)

	Monsoon					Summer				
Parameters	A	B	C	D	E	A	B	C	D	E
Temp (^{O}C)	50	56	58	51	50	52	58	61	56	54
pH	7.32	7.64	7.48	7.53	7.50	7.72	7.98	7.92	7.86	7.78

EC (mS cm^{-1})	5.71	5.86	5.99	5.76	5.96	1.66	1.79	1.74	1.68	1.71
DO (mg L^{-1})	4.98	5.41	5.37	5.28	5.28	7.44	7.63	7.38	6.98	6.89
Na^+ (mg L^{-1})	87.5	94	91	83.5	78.5	112.5	117	115.5	121.5	120
K^+ (mg L^{-1})	6.3	5.8	6.3	5.5	5.5	11.2	15.4	12.9	13.1	13.1
Ca^{++} (mg L^{-1})	2.6	3.1	2.6	2.25	2.25	0.9	1.1	0.8	0.9	0.8
Cl^- (mg L^{-1})	142.6	155.33	151.74	138.38	144.24	132.76	149.41	146.12	142.89	142.32
PO_4^{-3}(mg L^{-1})	-	-	0.01	0.02	0.04	-	-	-	-	-
NH_4^+ (mg L^{-1})	28.62	27.92	28.41	31.72	34.56	12.23	10.62	11.78	12.14	12.46
HCO_3 (mg L^{-1})	1185	1360	1325	1340	1280	510	525	490	490	480

Study of pigments:

Chlorophyll-a and carotenoid contents of the cyanobacterial samples, collected from five sites, have been measured in two seasons summer and monsoon. The results show variations in pigment contents site wise as well as season wise. In summer highest carotenoid content is 5.354 µg/ml in site B whereas highest chlorophyll-a content is 2.03 µg/ml in the same site. In monsoon season highest chlorophyll-a production is 4.97 µg/ml in site A while the content is very low (1.076 µg/ml) in summer in the same site. In monsoon production of carotenoids also decreases (0.654 to 0.4 µg/ml). Ratio of carotenoids and chlorophyll-a in these samples of the hot spring also show variation. The ratio is high in summer (highest 2.637 in site B) and lowest 1.578 in site D. In the monsoon season the ratio is lower (highest 0.227 in site B and lowest 0.132 in site A).(Table-3).

Table 3: Results showing seasonal variation in chlorophyll-a and carotenoids contents of cyanobacterial mats collected from different locations of Panifala hot spring

	Summer			Monsoon		
Locations	Carotenoid (µg/ml)	Chlorophyll-a (µg/ml)	Carotenoid:Chl-a ratio	Carotenoid (µg/ml)	Chlorophyll-a (µg/ml)	Carotenoid:Chl-a ratio
Site A	2.148	1.076	1.996	0.654	4.97	0.132
Site B	5.354	2.03	2.637	0.746	3.28	0.227
Site C	2.352	1.217	1.933	0.538	3.33	0.162
Site D	1.651	1.046	1.578	0.432	2.19	0.197
Site E	4.102	1.624	2.526	0.4	2.18	0.183

Discussion

Presence of cyanobacterial species in moderately large number in the hot springs is an example of tolerance of extreme habitats by this group of organisms. In geothermal springs temperature has been one of the most important factors as far as the distribution and diversity of cyanobacterial species is concerned. Significant decrease in cyanobacterial diversity with increasing temperature has been observed by Sompong *et al.* (2005) and Debnath *et al.* (2009). A temperature range of 50°C to 75°C favours the formation of layered mats by a true thermal unicellular taxon *Synechococcus* in almost all thermal springs around the world (Yoneda, 1952; Ferris *et al.*, 1996; Ferris and Ward, 1997; Ward and Castenholz, 2002). Cyanobacterial mats occurring at the lower end of thermophily (40-50°C) often comprise dominant filamentous members like *Fischerella, Calothrix, Oscillatoria, Phormidium and* non-filamentous *Gloeocapsa* (Ward and Castenholz, 2002; Sompong *et al.*, 2005). A very similar situation has been observed in the present survey also. In Panifala, mats from a thermal gradient of 50-61°C are dominated by species of *Synechococcus* (54-61°C) and *Phormidium* (50-56°C). *Phormidium tenue, P. laminosum* and *Calothrix braunii* are few facultative mesothermophilous species prevailing in sites with relatively lower temperature range (50-56°C). True thermal species like *Synechococcus elongatus, Fischerella thermalis and Calothrix* *thermalis* which are found in almost all thermal springs (Yoneda, 1952; Castenholz, 1969; Kastovsky and Komarek, 2001; Debnath *et al.,* 2009) are also found here. Results obtained from relative species abundance evidently show that high temperature species like *S. bigranulatus S. elongatus* are abundant in site B (56-58°C) and site C (58-61°C) in both sampling seasons. High relative abundance of *P. tenue, P. laminosum* and *F. thermalis* has been observed in relatively lower temperature zones like site A (50-52°C), site D (51-56°C) and site E (50-54°C). Besides relative abundance of *P. tenue, P. laminosum, F. thermalis, C. minutus, Gloeocapsa* sp. is lower in sites having water temperature at or above 56°C. *Scytonema millei* has been found to co-occur with *Phormidium* sp. in sites A, D and E where water temperature ranges between 50-56°C. This kind of species distribution pattern along temperature difference is highly similar to the findings of Debnath *et al.* (2009) as observed from Bakreswar geothermal springs.

Works of Ward and Castenholz (2002), Sompong *et al*. (2005) have emphasized the role of pH and NH_4^+content on species distribution and diversity in cyanobacterial mats. Cyanobacterial mats of Panifala thermal spring comprise a good assemblage of members of Chroococcales, Oscillatoriales, Nostocales and Stigonematales though maintaining fairly higher amount of ammonium content (27.92-34.56 mg L^{-1}) in monsoon and 10.62-12.46 mg L^{-1} in summer. In site B, species occurrence is high as compared to other sites in both the sampling seasons. Though there are variations in pH and NH_4^+content between two seasons the species diversity and occurrence is the same. In this site highest pH (7.64 in monsoon; 7.98 in summer) and lowest NH_4^+content (27.92 mg L^{-1} in monsoon; 10.62 mg L^{-1} in summer) has been observed in both sampling seasons. In site E the water samples have highest NH_4^+content (34.56 mg L^{-1}) and relatively lower pH (7.50-7.78) among the collected water samples. The relative abundance of various species is low here and the non-heterocystous members like species of *Synechococcus, Chroococcus and Phormidium* have been observed in this site. This observation evidently supports the findings of Ward and Castenholz (2002). Debnath *et al.* (2009) also observed highest species number in a sampling site having lowest amount of NH_4^+ content (0.08 mg L^{-1}) and lowest number in site having highest NH_4^+ content (0.46 mg L^{-1}).

Comparing the species composition from geothermal springs of Bakreswar reported by Debnath *et al.* (2009) with the present study, we find a variation in species composition between these two hot spring locations. There is also some variation in physico-chemical parameters of water between these two locations. Water of Panifala exhibits relatively much higher level of NH_4^+ (27.92-34.56 mg L^{-1}) while in Bakreswar the concentration of the same is 0.06-0.46 mg L^{-1}. Electrical conductivity in Panifala is 1.66-5.99 mS cm^{-1} while that in Bakreswar is 0.375-0.499 mS cm^{-1}. Concentration of Cl^- is 132.76-155.33 mg L^{-1} in Panifala whereas in Bakreswar the concentration is 19.2-23.1 mg L^{-1}. However level of Na^+, K^+ and Ca^{++} content is lower in Panifala as compared with that of Bakreswar hot springs. These differences in water chemistry along with temperature variation (50-61°C in Panifala whereas in Bakreswar it is 38-60°C) are the key factors that led to somewhat different species composition in two above mentioned geothermal areas. While *Phormidium tenue, Calothrix braunii* and *Scytonema millei* are the collected species in Panifala which have not been found in Bakreswar, species of *Oscillatoria, Pseudanabaena* and *Chlorogloeopsis* are completely absent in Panifala.

In temperature range of 51 to 56°C the increase in the standing crop in hot spring environment is indicated by the production of high amount of chlorophyll-a (Fraleigh and Wiegert, 1975). But there are some contrasting views regarding the relation among gross production, chlorophyll-a content and light intensity. The rate of gross production is directly related to chlorophyll-a content but independent of light intensity (Fraleigh and Wiegert, 1975). On the other hand, Brock (1967) has stated that photosynthetic rate per unit chlorophyll is dependent on light intensity. It can be assumed that the light attenuates greatly within the cyanobacterial layer, leaving many cells photosynthetically unsaturated. Thus, a greater percentage of cells in thinner mats become light saturated. Increased production of carotenoids in summer season over that of chlorophyll-a may reflect a fact that some carotenoids in microorganisms protect cell constituents from photooxidations sensitized by chlorophyll-a and other pigments (Krinsky, 1966). The protective role of carotenoids against higher light and temperature during summer is well established. In our present experiment elevated level of carotenoids production (5.354 µg/ml) over that of chlorophyll-a (2.03 µg/ml) has been observed in summer season. Carotenoid:chl-a ratio rises upto 2.637 (in site B) during this season whereas in monsoon carotenoid: chl-a ratio falls upto 0.132 (in site A) which is lowest among five sampling sites.. This evidently supports the protective role of carotenoids to prevent photodesturction of chlorophyll-a in higher light and temperature regime. Higher expression of chlorophyll-a from monsoon samples (highest 4.97 µg/ml in site A) than that of carotenoids (highest 0.746 µg/ml in site B) indicates seasonal variation of chlorophyll-a and carotenoids in cyanobacterial mats. Further Eco-physiological studies are required to understand the adaptability of these thermophilic species of cyanobacteria.

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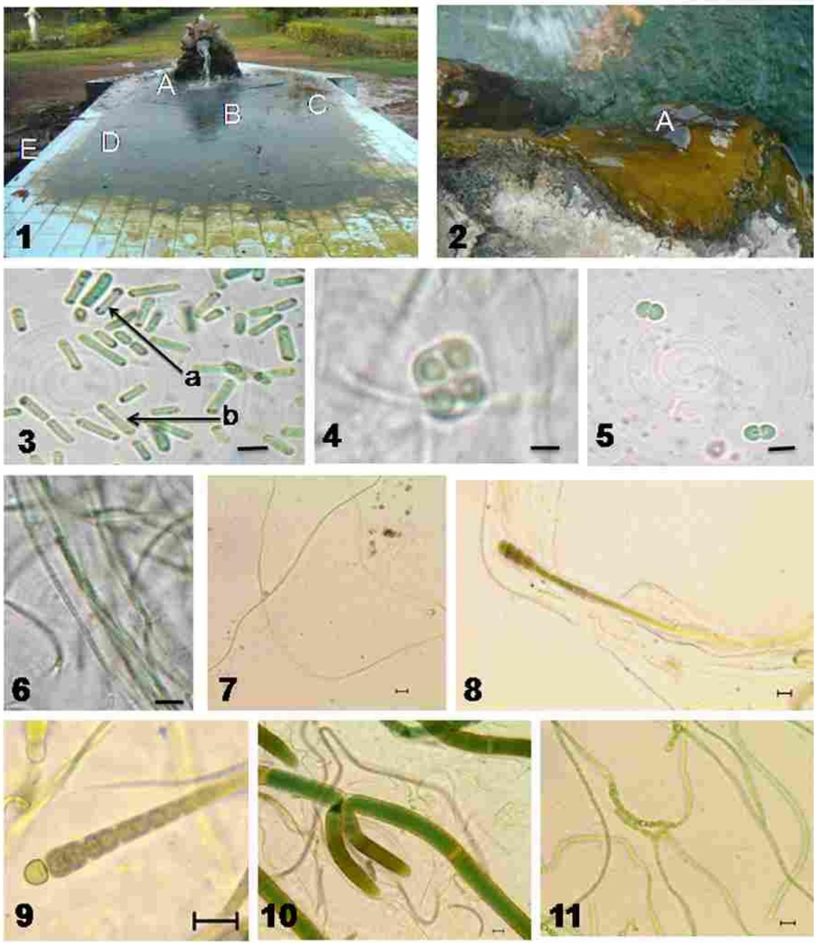

Plate 1. Sampling site and the Cyanobacteria collected. Fig. 1. Sampling sites (A, B, C, D and E); fig. 2. Cyanobacterial mats (A) of various colours; fig.3. Strains of a) *Synechococcus bigranulatus* and b) *S. elongatus;* fig. 4. *Chroococcus minutus* in four celled stage; fig. 5. *Gloeocapsa* sp. forming two celled stage; fig. 6. *Phormidium tenue*; fig. 7. *P. laminosum;* fig. 8. *Calothrix thermalis*; fig. 9. *Calothrix braunii;* fig. 10. *Scytonema* sp. showing false branching; fig. 11. *Fischerella thermalis*; Scale bars: figs. 3, 4, 6, 7 = 5 μm, figs. 5, 8, 9, 10, 11 = 10 μm.

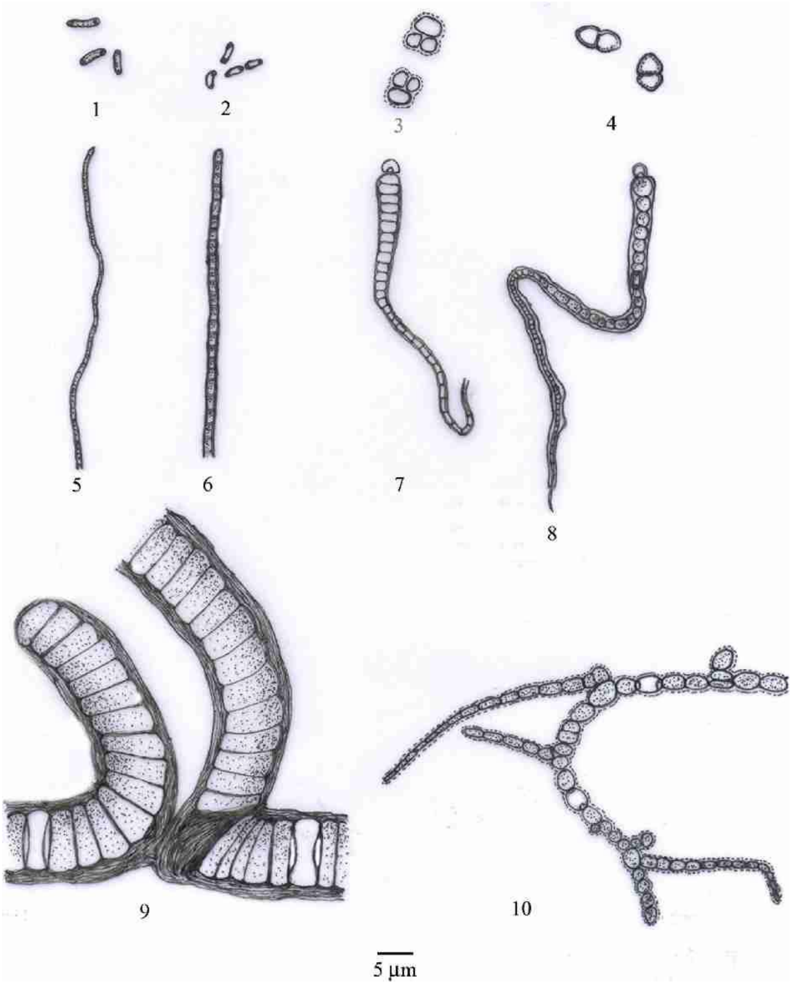

Plate 2. Camera-Lucida drawing of collected thermophilic cyanobacterial species. Fig. 1. *Synechococcus elongatus;* fig.2. *Synechococcus bigranulatus;* fig. 3. *Chroococcus minutus;* fig. 4. *Gloeocapsa* sp.; fig. 5. *Phormidium laminosum;* fig. 6. *Phormidium tenue;* fig. 7. *Calothrix braunii ;* fig. 8. *Calothrix thermalis;* fig. 9. *Scytonema millei* (from culture)*;* fig. 10. *Fischerella thermalis* (from culture).