

Cyanobacterial diversity of two hyper-thermal springs, Ringigad and Soldhar in Tapoban geothermal field, Uttarakhand Himalaya

The Indian Himalayan Region (IHR) is characterized by the presence of some extreme environments, such as thermal springs and glaciers, and thus provides an opportunity to study the extremophilic microbial diversity. A thermal gradient had developed in the thermal springs along the run-off channel down the gradient, which was maintained throughout the year. Under the influence of this thermal gradient, an assemblage of microorganisms takes place according to their thermal tolerance. This results in the development of a colourful microbial mat along the run-off channel.

Tapoban, a prominent geothermal field in the Uttarakhand Himalaya, has received attention for conducting studies on thermophiles (Figure 1). Studies on cyanobacterial diversity in low-temperature thermal springs (42–50°C) have been carried out earlier^{1,2}. Recently, Trivedi *et al.*³ reported work on diversity of thermophilic eubacteria and yeast associated with two hyper-thermal springs, Ringigad and Soldhar (drill-holes AGW-2 and AGW-3 respectively, drilled by the Geological Survey of India), in the Tapoban geothermal field, Uttarakhand Himalaya. In this context, it has been observed that in spite of the presence of hyper-thermal springs in the IHR, reports on their thermophilic cyanobacteria do not exist, except in Jamnotri². Besides, a power generation project has been proposed in the Tapoban geothermal field. Since microbial diversity is vulnerable and may face at least local extinction due to anthropogenic activities, its documentation is of utmost importance. Due to their metabolic flexibility and adaptability, cyanobacteria are getting recognition in recent times for their potential applications^{4,5}. Therefore, the present study was aimed to assess the cyanobacterial diversity of two hyper-thermal springs, with special reference to the thermal tolerance. The data generated in the present study will be of use for further research and development in the Indian context.

Based on the thermal gradient, cyanobacterial mat samples were collected from four ranges (30–40°C, 40–50°C, 50–60°C and 60–70°C), in thoroughly washed and autoclaved polypropylene

sample bottles (Tarson, India). The samples were immediately preserved in 4% formalin, and one set was kept separately for enrichment studies. Enrichment of the cyanobacterial component of the thermal springs was done using media-D (ref. 6) prepared in about 25% of geothermal water. This method essentially comprises the conditions which more or less mimic the natural environment. Next, 1 ml of the homogenized pooled sample was added to the enrichment flasks, and incubated at 45°C and 55°C (Remi Incubator, India), and 14/10 h light and dark cycles with illumination of 2000 lux by cool fluorescent tube (Philips, India) for 15–20 days.

The microscopic observations were carried out at 400× and 1000× magnification using compound light microscope (Olympus CH20i). Taxonomic criteria were followed as given in the literature^{7–11}. The taxonomic descriptions provided in the literature^{12–16} were used for species identification. Photomicrographs of different taxa identified during the study were taken using Magnüs MIPS-USB (Olympus).

A total of 31 thermophilic cyanobacterial species representing three orders, nine families and 13 genera were recorded from both the thermal springs (Table 1; Figure 2). A total of 24 species belonging to Chroococcales (8; 33.33%), Oscillatoriales (15; 62.5%) and Stigonematales (1; 4.16%) were recorded at Soldhar within the temperature range 30–70°C. Similarly, a total of 22 species

belonging to Chroococcales (3; 13.63%), Oscillatoriales (17; 77.27%) and Stigonematales (2; 9.09%) were recorded at Ringigad within the temperature range 30–70°C. Surprisingly, the Nostoclean genera were absent in both the thermal springs. In contrast, the Nostoclean genera such as, *Microchaete*, *Calothrix*, *Tolypothrix* and *Anabaena* have been reported from Badrinath, Tapoban, Jamnotri and Gaurikund thermal springs^{1,2}. However, except Nostocales, the occurrence of different orders of cyanobacteria in Ringigad and Soldhar is in coherence with earlier studies^{1,2,6,14,17,18}.

A survey of the literature indicates that some of the taxa observed in the present study, have been reported from other Indian thermal springs. To the best of our knowledge, taxa such as *Chroococcus turgidus* (Kütz) Nägeli, *C. tenex* (Kirchn) Hieron., *Synechocystis sallensis* Skuja, *Gloeocapsa livida* (Carm.) Kütz, *Myxosarcina* sp., *Hydrococcus rivularis* Kütz, *Pseudanabaena galeata* Anag., *Oscillatoria animalis* Ag. ex Gomont, *O. pseudogeminata* G. Schmid var. *Unigranulata* Biswas, *O. simplicissima* Gomont, *O. cruenta* Grun., *O. chlorina* Kütz. ex Gomont, *O. princeps* Vaucher ex Gomont, *Phormidium bohaneri* Schmidle, *P. cebennense* Gomont, *Lyngbya hieronymusii* Lemm., *Spirulina subsalsa* Oerst. ex Gomont, *Spirulina meghiniana* Zanard. ex Gomont, and *Chlorogloeopsis* sp. are the new records for thermal springs of Uttarakhand Himalaya. Of these taxa, *P. galeata* Anag.,

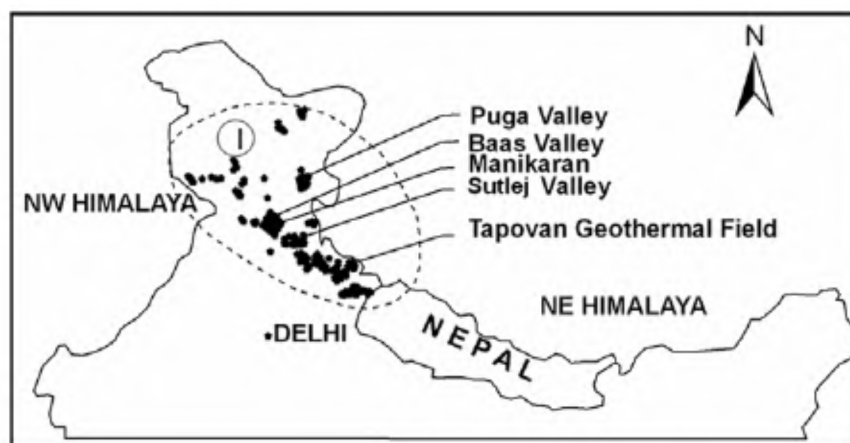


Figure 1. Location map of Tapoban geothermal field.

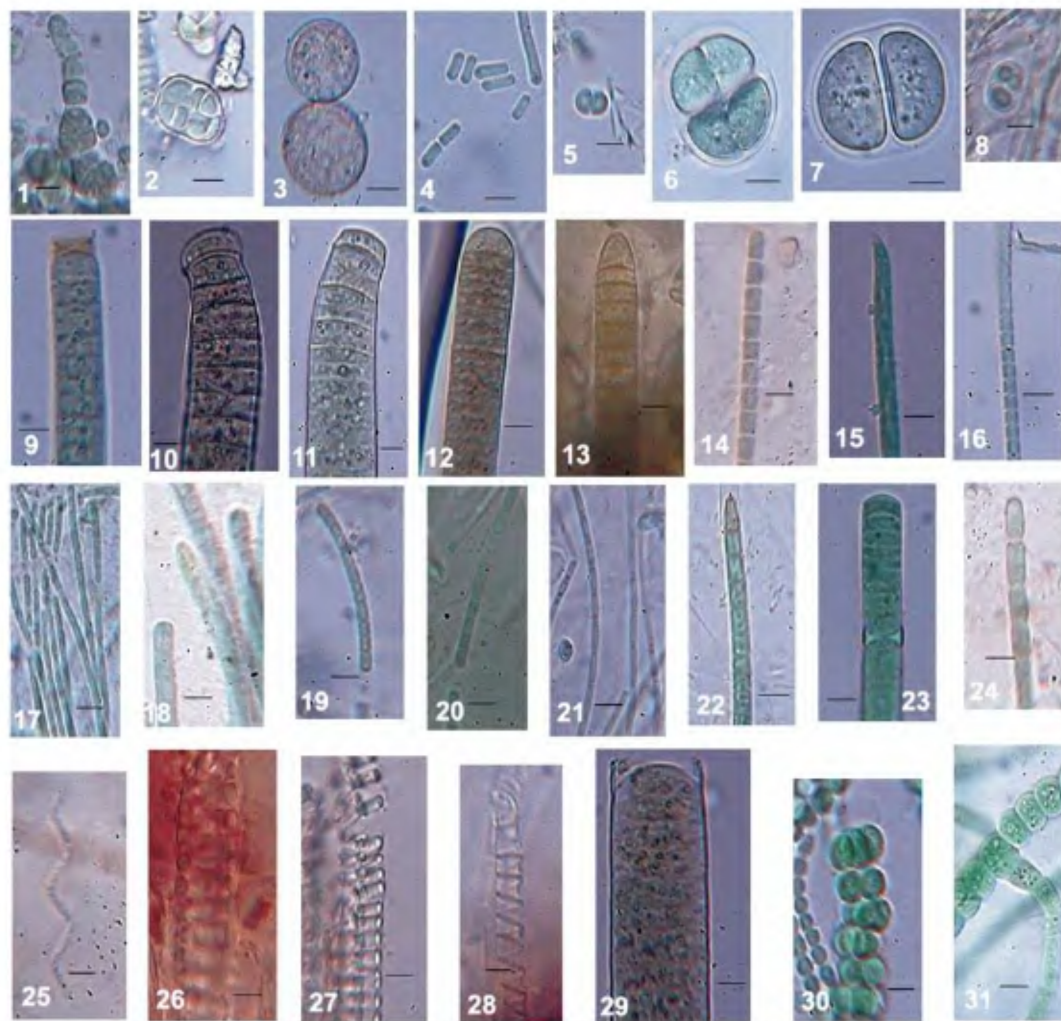


Figure 2. Microscopic features of cyanobacterial morphotypes in the present study. (1) *Hydrococcus rivularis* Kütz.; (2) *Myxosarcina* sp.; (3) *Synechocystis sellensis* Skuja; (4) *Synechococcus elongatus* Nägeli; (5) *Gloeocapsa livida* (Carm.) Kütz.; (6) *Chroococcus turgidus* (Kütz) Nägeli; (7) *C. tenax* (Kirchn) Hieron.; (8) *C. minimus* (Keissl.) Lemm.; (9) *Oscillatoria simplicissima* Gomont; (10) *O. proboscidea* Gomont; (11) *O. princeps* Vaucher ex Gomont; (12) *O. limosa* Ag. ex Gomont; (13) *O. cruenta* Grun.; (14) *O. chlorina* Kütz. ex Gomont; (15) *O. animalis* Ag. ex Gomont; (16) *Pseudanabaena galeata* Anagnostidis; (17) *Phormidium frigidum* Fritsch.; (18) *P. corium* (Ag.) Gomont; (19) *P. cebennense* Gomont; (20) *P. bohneri* Schmidle; (21) *P. africanum* Lemm.; (22) *P. abronema* Skuja; (23) *Oscillatoria subbrevis* Schmidle; (24) *O. pseudogeminata* G. Schmid var. *unigranulata* Biswas; (25) *Arthrospira tenues* Bhur et Biswas; (26) *Spirulina* sp.; (27) *S. subsalsa* Oerst. ex Gomont; (28) *S. meneghiniana* Zanard. ex Gomont; (29) *Lyngbya hieronymusii* Lemm.; (30) *Chlorogloeopsis* sp., and (31) *Mastigocladus laminosus* (Fischerella) Cohn (bar = 5 µm).

L. hieronymusii Lemm. and *Chlorogloeopsis* sp. are new records for Indian thermal springs.

Not all the temperatures are equally suitable for the growth and reproduction of living organisms. The occurrence of the species in thermal springs is likely to be determined by the respective thermal gradients. Most of the cyanobacterial species were recorded in the temperature range 35–40°C, followed by 40–50°C, 50–60°C and 60–70°C in Soldhar and Ringigad (Table 1). In Ringigad, a total of 21 species were recorded at 30–40°C, which was followed by 17 at 40–50°C, 7 at 50–60°C and 5 at 60–70°C. Similarly,

in Soldhar, a total of 23 species were recorded at 30–40°C followed by 16 at 40–50°C, 9 at 50–60°C and 5 at 60–70°C. Earlier studies have reported the occurrence of a number of cyanobacterial taxa up to 80 or even 84°C (refs 1, 2, 17, 18). However, we could not observe the occurrence of thermal cyanobacteria up to such high temperatures.

When the microbial mat collected from 60°C to 70°C was allowed to grow under enrichment at similar temperatures, no growth was observed. Even *Synechococcus elongatus*, which was observed up to 70°C, was unable to grow at this temperature range under enrich-

ments. It is an indicative of the fact that various ecological factors such as unidentified crucial nutritional components, heat-shock proteins¹⁹, biological interactions or cell-to-cell signalling²⁰ work together for proper physiological functions at elevated temperatures under natural conditions, which could not be provided under artificial culture condition. Efforts are being made to culture and preserve the reported cyanobacteria in our laboratory. Ecological studies, isolation in mixed or axenic form and application of molecular tools are likely to give a better insight into the adaptation mechanisms under natural and artificial

Table 1. Cyanobacterial diversity and its distribution along the thermal gradient

Taxa	Soldhar (AGW-3)	Ringigad (AGW-2)	30–40°C	40–50°C	50–60°C	60–70°C
<i>Chroococcus minimus</i> (Keissl.) Lemm.	+	+	+	+	+	–
<i>Chroococcus turgidus</i> (Kütz.) Nägeli	+	–	+	–	–	–
<i>Chroococcus tenex</i> (Kirchn.) Hieron.	+	–	+	–	–	–
<i>Synechococcus elongatus</i> Nägeli	+	+	–	+	+	+
<i>Synechocystis sellensis</i> Skuja	+	+	+	+	–	–
<i>Gloeocapsa livida</i> (Carm.) Kütz	+	–	+	+	–	–
<i>Hydrococcus rivularis</i> Kütz	+	–	+	+	–	–
<i>Myxosarcina</i> sp.	+	–	+	+	–	–
<i>Oscillatoria animalis</i> Ag. ex Gomont	+	+	+	+	+	–
<i>Oscillatoria limosa</i> Ag. ex Gomont	+	+	+	–	–	–
<i>Oscillatoria pseudogeminata</i> G. Schmid var. <i>unigranulata</i> Biswas	–	+	+	+	–	–
<i>Oscillatoria subbrevis</i> Schmidle	+	–	+	+	–	–
<i>Oscillatoria chlorina</i> Kütz. ex Gomont	+	+	+	+	–	–
<i>Oscillatoria simplicissima</i> Gomont	–	+	+	–	–	–
<i>Oscillatoria proboscidea</i> Gomont	–	+	+	–	–	–
<i>Oscillatoria cruenta</i> Grun.	+	+	+	+	–	–
<i>Oscillatoria princeps</i> Vaucher ex Gomont	–	+	+	–	–	–
<i>Phormidium africanum</i> Lemm.	+	+	+	+	+	+
<i>Phormidium frigidum</i> Fritsch.	+	+	+	+	+	+
<i>Phormidium bohneri</i> Schmidle	+	+	+	+	+	+
<i>Phormidium cebennense</i> Gomont	+	+	+	+	+	+
<i>Phormidium abronema</i> Skuja	+	+	+	+	–	–
<i>Phormidium corium</i> (Ag.) Gomont	–	+	+	–	–	–
<i>Lygbya hieronymusii</i> Lemm.	+	+	+	+	–	–
<i>Arthrospira tenuis</i> Bhrul et Biswas	–	+	+	–	–	–
<i>Spirulina</i> sp.	+	+	+	+	–	–
<i>Spirulina subsalsa</i> Oerst. ex Gomont	+	–	+	+	–	–
<i>Spirulina menghiniana</i> Zanard. ex Gomont	+	–	+	+	–	–
<i>Pseudanabaena galeata</i> Anagnostidis	+	–	+	+	–	–
<i>Mastigocladus laminosus</i> Cohn	+	+	+	+	–	–
<i>Chlorogloeopsis</i> sp. (only in enrichment)	–	+	+	+	–	–

+, Presence; –, Absence.

growth conditions. In the context of thermal springs of IHR, the observations noted in the present study will help in defining the future research prospects.

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