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Cyanobacterial community structure in hot water springs of Indian North-Western Himalayas: A morphological, molecular and ecological approach

Yadvinder Singh[a,1,2], Arvind Gulati[b,2], D.P. Singh[a], J.I.S. Khattar[a,*]

[a] Department of Botany, Punjabi University, Patiala 147002, Punjab, India
[b] Microbial Prospection Division, CSIR-Institute of Himalayan Bioresource Technology, P.O. Box 6, Palampur 176 061, Himachal Pradesh, India

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ABSTRACT

Cyanobacterial diversity from nine hot water springs of North-Western Himalayas scattered over an area of approximately 20,000 km^2 has been studied using polyphasic approach in relation to important ecological factors. These hot springs have hard water, with variation of temperature from 40 to 90 °C and pH from 6.8–8.0. A total of 625 cyanobacterial isolates were cultured from 150 samples. On the basis of morphology, these isolates were represented by 22 species of 11 genera. A total of 220 cyanobacterial isolates representing morpho-species from each sampling site were subjected to molecular characterization by amplified ribosomal DNA restriction analysis (ARDRA), 16S rRNA gene, *rbcL* gene and *cpcB*-IGS-*cpcA* phycocyanin locus region sequencing for elucidating their identity, diversity and phylogenetic relationships. ARDRA analysis revealed 22 groups of cyanobacterial isolates from selected hot water springs. Sequence analysis of 16S rRNA gene, *rbcL* gene and *cpcB*-IGS-*cpcA* phycocyanin locus region revealed that the identity of the majority of the cyanobacterial species was congruent with the identity based on morphological features. The identity of 3 taxa; *Leptolyngbya* sp. PUPCCC 112.22, *Phormidium* sp. PUPCCC 118.2 and *Phormidium* sp. PUPCCC 118.3, based on both morphological features and molecular markers, did not match with known cyanobacterial species, indicating these as new genera/species. Phylogenetic analyses revealed that taxa from selected hot springs belonging to Stigonematales are monophyletic, whereas Chroococcales and Oscillatoriales are polyphyletic. Canonical correspondence analysis of physico-chemical parameters and cyanobacterial community of each hot water spring revealed that temperature, pH, conductivity, nitrogen, sulphate and phosphate influenced cyanobacterial community structure. Further, our study has revealed that the cyanobacterial community of hot water springs is comprised of endemic as well as globally distributed representatives and is influenced by ecological parameters. The nature of the cyanobacterial community structure depended on the water chemistry rather than geographical location of the spring.

1. Introduction

Cyanobacteria, oxygen-producing photosynthetic prokaryotes, are an integral part of most of terrestrial and aquatic ecosystems. These organisms are a dominant microbial community of most of extreme environments including hot springs, bare rocks, cold environments and desert soils [1]. Hot water springs are widely distributed across the globe with water temperature significantly above the mean of annual air temperature of that region to boiling point of water [2]. These springs represent well-isolated unique habitats in globally distant regions. The microorganisms inhabiting these waters are well adapted to conditions quite different from the surrounding ambient environment through which they have to disperse [3]. The unique adaptations of the microbial flora to such harsh environments have long attracted the attention of microbial ecologists [4].

Some cyanobacterial strains in association with microbial mats are able to thrive in the thermal springs at 74 °C, upper temperature limit for photosynthesis [5]. Thermophilic cyanobacteria from the Yellowstone National Park were the first, and are the possibly the most extensively studied organisms [6]. Although cyanobacteria from some other thermal springs have also been studied to some extent, many such habitats still remain unexplored [7–9]. From India, only few reports on cyanobacterial diversity from hot water springs are available, which include studies on thermal springs of western part of the country by Thomas & Gonzalves [10]; from Bihar [11,12], from Uttarakhand [13] and from West Bengal [14–18].

* Corresponding author.
E-mail address: jisk_pbi@rediffmail.com (J.I.S. Khattar).
[1] Present address: Department of Botany and Environmental Science, Sri Guru Granth Sahib World University, Fatehgarh Sahib-140406, Punjab, India.
[2] Equal contribution to the work.

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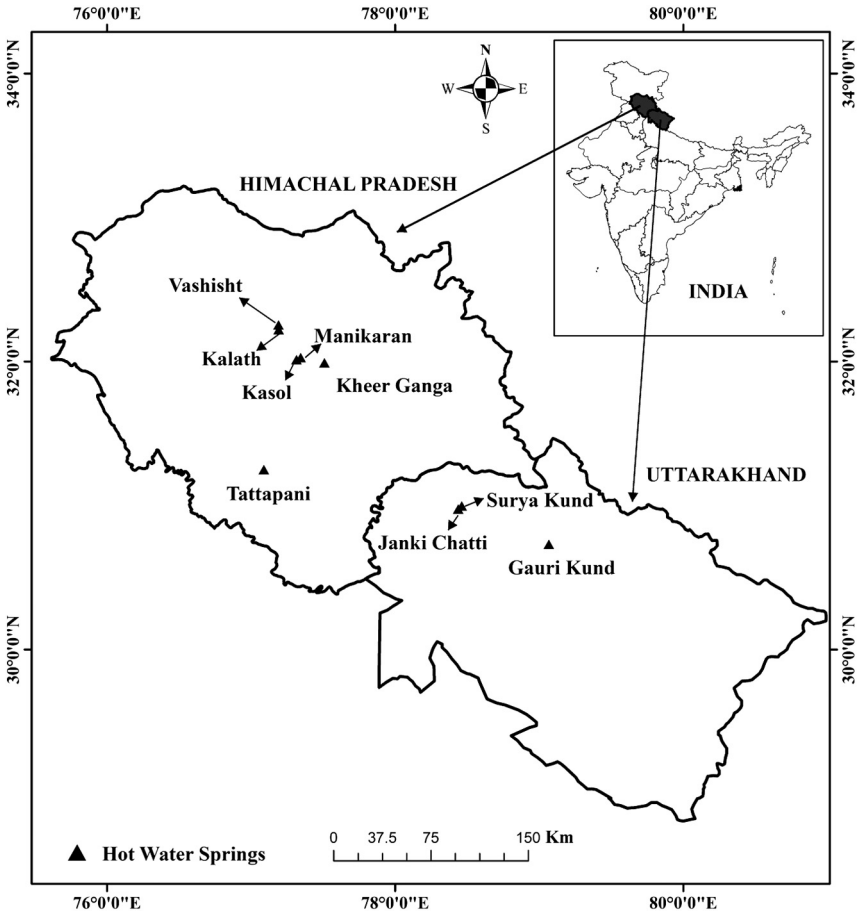

Fig. 1. Location of hot water springs of North-Western Himalayas.

The North-Western Himalayas are one of the major bio-geographical zones of India, represented by diverse microenvironments and unique ecosystems. Hot springs are one of the unique biotopes of this region. Out of 340 hot springs spread all over India, 62 are distributed along the North-Western Himalayas, in the states of Uttarakhand, Jammu & Kashmir and Himachal Pradesh [19]. These springs are mostly along the river valleys and are concentrated along a 30–50 km wide thermal belt. The hot water springs of Himachal Pradesh and Uttarakhand belong to tectonic belts of the Himalaya geothermal province. Some of the hot springs from this area have been developed for recreational and tourism purposes by uplifting the water from the spring, while others are located in remote areas, are completely undisturbed and are free from direct anthropogenic activities. Previous studies of the Himalaya geothermal province were mainly focused on the famous thermal springs of Manikaran and Kasol, in Himachal Pradesh, to characterize the geothermal resources with respect to their suitability for electric power generation [20,21]. Other workers focused their study on radon monitoring in water and soil for health hazard assessment and as a tool for earthquake prediction [22–25]. A few studies have reported chemical [26] and isotopic data [27] of the thermal waters.

Thermophilic microorganisms are interesting scientifically for their analogy to the ancient life forms on Earth and are valuable as a source of thermostable biomolecules. The cyanobacterial diversity of hot water springs of North-Western Himalayas remained unexplored and needs immediate attention. Isolation, purification, taxonomic characterization and axenic cultivation of thermophilic cyanobacterial strains may prove to be a good source of novel organisms for possible biotechnological exploitation for thermostable enzymes, proteins and pigments [28]. In this context, enough attention has not been given to the thermophilic cyanobacterial germplasm of Indian geothermal springs. The aim of the present study was to investigate the morphological, molecular and ecological diversity of thermophilic cyanobacteria in relation to physico-chemical parameters of hot water springs of North-Western Himalayas and to bring them into axenic cultures for characterization and possible exploitation.

2. Materials and methods

2.1. Study sites and sampling

Nine hot water springs, 6 in Himachal Pradesh (Tattapani, Manikaran, Kheer Ganga, Vashisht, Kasol and Kalath) and 3 in Uttarakhand (Surya Kund, Janki Chatti and Gauri Kund) scattered over an area of approximately 20,000 km^2 from an altitude of 675 to 3505 m were investigated (Fig. 1). Water samples from these sites were collected in pre-sterilized 1 L polyethylene bottles for chemical analysis. The water samples were filtered through 0.45-μm pore-diameter membranes and acidified (1% HNO_3, v/v). Biological mats, concretions and sediments were randomly picked with sterile forceps and spatula from the sampling sites and placed in sterile glass containers. Water sample for planktonic cyanobacterial strains were collected in sterile glass vials and tubes.

2.2. Physico-chemical parameters of water

Water samples from each sampling site were subjected to physico-chemical analysis. Water temperature was noted with a thermometer at the site of collection. The pH of the water at the site was noted by a digital pH meter (Orion, Thermo Fisher Scientific, Waltham, MA, USA) and conductivity was determined by a conductivity bridge (Systronics, Norcross, GA, USA). Water samples were analyzed in the laboratory for