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Cyanobacterial diversity along altitudinal gradient in Eastern Himalayas of India

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Abstract

Present study describes the cyanobacterial diversity at different altitudes viz. 300, 1500 and 3500 m asl in Arunachal Pradesh, the Eastern Himalayan region of India. Results showed that heterocystous forms reduced to 2 at 3500 m asl from 5 and 11 at 1500 and 300 m asl respectively. Unicellular forms were represented by 6 and 1 species at 300 and 1500 m asl respectively while they were not recorded at 3500 m asl. The numbers of non-heterocystous species were 5, 6 and 3 respectively at 300, 1500 and 3500 m asl. At low altitude of 300 m asl, unicellular and heterocystous species were more numerous, whereas at 3500 m asl non-heterocystous species were prevalent. This study showed that cyanobacterial species diversity is negatively correlated with acidity and altitude.

Key words: altitude; cyanobacteria; diversity; Eastern Himalayas; pH.

Introduction

Environmental degradation and their negative effects on productivity and fertility of ecosystems have necessitated the need of documentation of biosystems for sustainable practices. Cyanobacteria - photosynthetic prokaryotes - representing wide diversity in their forms and habitats are getting attention for three B's viz. Biofertilizer, Bioremediation and Bioactive compounds. During course of evolution, they diversified their forms from unicellular to filamentous, and extended their habitats from aquatic to terrestrial, acidophilic to alkalophilic, hot springs to extreme cold of Antarctic and Arctic, rock surfaces to desert and mountains (Sanchez-Baracaldo *et al*. 2005). Cyanobacteria are popularly exploited as biofertilizers for augmenting the content of nitrogen, phosphorus and other growth promoting substances along with some polysaccharide materials of the soil. Interest in cyanobacteria is mounting rapidly for their significant contribution in degradation of pollutants (Micheletti *et al*. 2008), production of biomolecules of pharmaceutical and nutraceutial value (Linington *et al.* 2007) and biofuel production (Hu *et al*. 2008). In view of their numerous ecological, economical and biotechnological importances, documentation and bioprospecting of cyanobacterial diversity across the earth is urgently required (Purvis & Hector 2000).

The successful implementation of cyanobacterial biotechnology programme requires a concerted effort in exploring the diversity of cyanobacteria in diverse habitats in order to preserve them in a metabolically stable condition. Moreover, a basic understanding of factors governing patterns of microbial diversity is an important aspect of maintaining a viable biotechnology program. Several studies have reported qualitative and quantitative distribution pattern of cyanobacteria throughout the world in diverse habitats including different regions of India (Kemprai 2013; Choudhary 2009, 2011). An extensive study on the cyanobacterial species diversity along an altitudinal gradient in the Eastern Himalayan region is still lacking. In the present study, we proposed to analyze the cyanobacterial community composition of three different altitudes of Arunachal Pradesh (Itanagar, Ziro and Tawang) lying in the Eastern Himalayan region.

Materials and Methods

Study sites

Arunachal Pradesh is located at the latitude of $26^0 28'$N and $29^0 30'$N and longitude of $91^0 30'$E and $96^0 30'$E. This Northeastern Indian province, bounded from three sides, North, East and West by China, Myanmar and Bhutan respectively, has varied altitudinal and climatic conditions and harbors all major forest types. Three sites viz. (i) the capital town Itanagar (Papum Pare district), (ii) Ziro (Lower Subansiri district) and (iii) Tawang (Tawang district) located at different altitudes were selected for the study of cyanobacterial distribution along the altitudinal gradient.

Itanagar is situated at an altitude of 300 m asl between $26^0 55'$ and $28^0 21'$N latitude and $92^0 40'$ and $94^0 21'$E longitude with Kurung Kumey district in the north, Lower Subansiri district in the east, East Kameng district in the west and Assam in the south. The districts headquarter of Lower Subansiri district named Ziro is situated at an altitude of 1500 m asl between $27^0 37'$N latitude and $93^0 49'$E longitude. Ziro is bounded on the north by China and Upper Subansiri district of Arunachal Pradesh, on the south by Papumpare district of Arunachal Pradesh and Assam and, on the east by West Siang and some part of the Upper

Subansiri and on the west by East Kameng district of Arunachal Pradesh. Tawang is situated at 3500 m asl between $27^0 22'$ and $27^0 45'$N latitude and $90^0 15'$ and $90^0 45'$E longitude on the Northwest extremity of Arunachal Pradesh bounded by Tibet in the north, Bhutan in the south-west and West Kameng district in the east side.

Climatic condition

The three sites selected for documentation of cyanobacterial diversity differ in annual rainfall and temperature. The climate of Itanagar is humid and moist with annual rainfall of ca 400 mm and temperature ranging between 10-30^{0}C, the annual rainfall and temperature at Ziro is 1080 mm and 1.1 - 30.6^{0}C respectively, whereas the annual rainfall at Tawang is 2760 mm and temperature ranges between -5.5 ^{0}C - 29 ^{0}C.

Soils

Soils of Itanagar and Ziro is sandy loam in texture whereas soil of Tawang is classified as (i) rocky and loamy skeletal texture with soil depth shallow to medium, and (ii) sandy skeletal, loamy, fine loamy textured with soil depth medium to deep.

Analysis

pH (KCl) of the soil was measured in a 1:5 soil:water (w/v). Moisture content of the soil was calculated by reading the difference between initial weight (fresh weight) and oven dried weight of the soil at 105 ^{0}C for 48 h. Total nitrogen (%) content of soil was determined according to Piper (1966). Correlation coefficient was calculated using Microsoft Excel package and analyzed for their significance using Pearson's rank correlation.

Enumeration of cyanobacterial diversity

Documentation of cyanobacteria was performed with soil samples and heterogeneous biomass of cyanobacteria collected from three different altitudes. Sampling was done from July to August, 2004. Soils samples (100 g) were collected by removing upper 0.5 cm layer of soil from 5-6 randomly selected spots of 20 different locations of each study sites. Heterogeneous masses of cyanobacteria were collected from water bodies and attached to rock surfaces in 50 ml glass culture tubes containing BG-11 nutrient medium (Rippka *et al.* 1979). Sampling was done from both illuminated and shaded elevations. Soil samples were thoroughly mixed, air-dried at 25–35 ^{0}C and sieved. 10 g of each soil samples were transferred into 90 ml of BG-11 nutrient medium whereas soil suspension dilutions were plated onto solidified BG-11 medium. Small aliquots of cyanobacterial biomass collected from water bodies and masses attached to soil and rock surfaces were also transferred into nutrient medium and incubated under appropriate growth condition of light (14/10h of light/dark cycle and 3000 klux light intensity) and temperature (28 ± 2 ^{0}C). Cyanobacterial species appearing in the nutrient medium were observed under compound microscope and cyanobacterial species diversity was recorded. Finally, the number of cyanobacterial species belonging to unicellular, filamentous non-heterocystous and filamentous heterocystous forms was correlated with different altitudes.

Identification of cyanobacterial species was performed using morphological features such as cell size, shape, arrangement (unicellular, colonial or filamentous), morphology of the terminal cells and the presence or absence of heterocysts, akinetes, and gas vacuoles (Desikachary 1959).

Results

Thirty-five cyanobacterial species were recorded from collected field samples from Itanagar (300 m asl), Ziro (1500 m asl) and Tawang (3500 m asl) (Table 1). Out of 35, 15 were heterocystous, 13 non-heterocystous and 7 unicellular forms with 4 species of common occurrence among the sites (Table 1). Cyanobacterial species diversity decreased with increasing altitude (Table 2). The number of cyanobacterial species recorded at 300 m asl was 22 representing 11 heterocystous, 5 non-heterocystous and 6 unicellular forms. *Gloeocapsa alpina*, *Oscillatoria tenuis*, *Phormidium fragile* and *P. uncinatum* were dominant forms at 300 m asl. The samples collected from 1500 m asl contained 12 species of cyanobacteria represented by 5 heterocystous, 6 non-heterocystous and 1 unicellular form. Non-heterocystous forms of *Phormidium uncinatum* and *Oscillatoria subbrevis* dominated the region. The enumeration of cyanobacterial diversity from bare lands at 3500 m asl showed the presence of non-heterocystous filamentous forms (*Phormidium tenue, Phormidium autumnale* and *Oscillatoria* sp.) along with little representation of heterocystous forms (*Anabaena variabilis* and *Nostoc linckia*) growing in grasses on moist soil surfaces (Table 1). Correlation between the number of cyanobacteria and altitude was observed to be significantly negative (Fig. 1). Species number was dependent upon altitude and decreased with increase in altitude. Cyanobacterial species were distributed evenly and significantly different at different altitudes (R^2=0.774; $F_{1,59}$ altitude x No. of species = 198.25; $p<0.001$).

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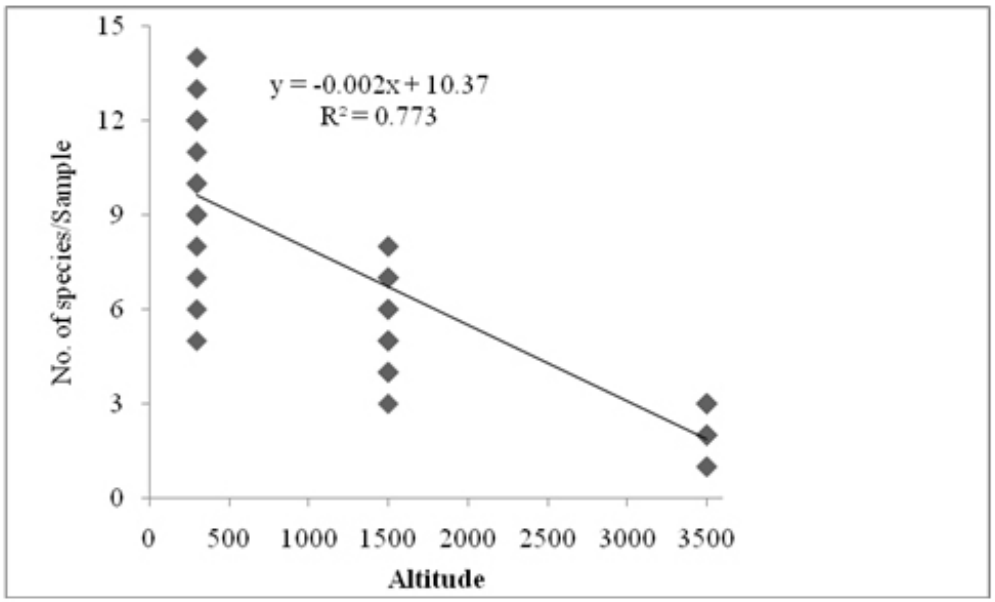

Figure 1. Scatter plot of cyanobacterial species diversity (No.) along three altitudes (r=0.880; p<0.001).

pH, moisture content, total nitrogen and number of cyanobacterial species at different altitudes is mentioned in Table 2. pH of the Itanagar (300 masl) soil was slightly acidic to neutral which became more acidic with increase in altitude. Total nitrogen content of the soil was maximum at 1500 m asl and minimum at 300 m asl. The study showed that the cyanobacterial species diversity was negatively correlated with acidity and altitude. In spite of more nitrogen at 1500 m asl, cyanobacterial species diversity was maximum at 300 m asl. Furthermore, 1500 m asl with high nitrogen content witnessed more heterocystous forms than 3500 m asl having low level of nitrogen.

Table 1. Cyanobacterial species recorded from Itanagar (300 m asl), Ziro (1500 m asl) and Tawang (3500 m asl) regions of Arunachal Pradesh. U - Unicellular; F - Filamentous; FH - Filamentous and Heterocystous; PH- Pseudobranched and Heterocystous and BH – Branched and Heterocystous.

S. No.	Species	Family	300 m asl	1500 m asl	3500 m asl
1	*Chroococcus turgidus* (Kütz.) Näg. (U)	Chroococcaceae	+	-	-
2	*Gloeocapsa alpina* (Näg) Brand (U)	Chroococcaceae	+	-	-
3	*G. kuetzingiana* Näg (U)	Chroococcaceae	+	-	-
4	G. *gelatinosa* (Menegh.) Kütz (U)	Chroococcaceae	+	-	-
5	*G. magma* (Bréb.) Kütz. (U)	Chroococcaceae	-	+	-
6	*Gloeothece rupestris* (Lyngb.) Bornet (U)	Chroococcaceae	+	-	-
7	*Chlorogloea fritschii* Mitra (U)	Entophysalidaceae	+	-	-
8	*Oscillatoria princeps* Vaucher ex Gomont (F)	Oscillatoriaceae	+	-	-
9	*O. tenuis* C. Agardh ex Gomont (F)	Oscillatoriaceae	+	-	-
10	O. *subbrevis* Schmidle *(F)*	Oscillatoriaceae	-	+	-
11	*Oscillatoria sp.* (F)	Oscillatoriaceae	-	-	+
12	*Phormidium fragile* Gomont (F)	Oscillatoriaceae	+	-	-
13	*P. lucidum* (C. Agardh) Kütz. ex Gomont (F)	Oscillatoriaceae	+	-	-
14	*P. uncinatum* (C. Agardh) Gomont ex Gomont (F)	Oscillatoriaceae	+	+	-
15	*P. tenue* (Menegh.) Gomont (F)	Oscillatoriaceae	-	-	+
16	*P. autumnale.* (C. Agardh) Gomont (F)	Oscillatoriaceae	-	-	+
17	*Lyngbya sp.* G.M. Smith (F)	Oscillatoriaceae	-	+	-

18	*Symploca muscorum* (C. Agardh) ex Gomont (F)	Oscillatoriaceae	-	+	-
19	*Microcoleus lacustris* (Rabenh.) Farlow ex Gom. (F)	Oscillatoriaceae	-	+	-
20	*M. chthonoplastes* Thuret ex Gomont (F)	Oscillatoriaceae	-	+	-
21	*Cylindrospermum stagnale* Kütz. Born. & Flah. (FH)	Nostocaceae	+	-	-
22	*C. muscicola* Kützing ex Born. & Flah. (FH)	Nostocaceae	-	+	-
23	*Nostoc calcicola Brébisson* ex Bornet & Flahault (FH**)**	Nostocaceae	+	-	-
24	*N. commune* Vaucher ex Born. & Flah. (FH)	Nostocaceae	+	+	-
25	*N. linckia* (Roth) Born. ex Born. & Flah. (FH)	Nostocaceae	-	-	+
26	*Anabaena sp.* (FH)	Nostocaceae	+	-	-
27	*Anabaena sp.* (FH)	Nostocaceae	+	-	+
28	*A. variabilis* Kützing (FH)	Nostocaceae	+	-	-
29	*A. oryzae* F.E. Fritsch (FH)	Nostocaceae	-	+	-
30	*Scytonema bohneri* Schmidle (PH)	Scytonemataceae	+	-	-
31	*Tolypothrix magna* Bharadwaja (PH)	Scytonemataceae	+	-	-
32	*Calothrix brevissima* G.S. West (PH)	Rivulariaceae	+	-	-
33	*C. marchica* Lemmermann (PH)	Rivulariaceae	+	+	-
34	*C. braunii* Born. & Flah. (PH)	Rivulariaceae	+	-	-
35	*Hapalosiphon welwitschii* W. West & G.S. West. (BH)	Stigonemataceae	-	+	-

Table 2. Cyanobacteria species richness with respect to pH, moisture and total nitrogen content of the soil along altitudinal gradients.

Location	Elevation (m asl)	pH	Moisture	Cyanobacterial Species	Total Nitrogen (%)
Itanagar	300	6.8 ± 0.3	19.23	22	0.83
Ziro	1500	6.4 ± 0.2	28.62	12	1.22
Tawang	3500	5.6 ± 0.2	48.35	05	0.91

Discussion

Comparative analysis of cyanobacterial species diversity showed a sharp decrease with increase in altitude. This might be attributed to diverse climatic conditions particularly precipitation, irradiance, pH, nutrient status and acidity occurring along altitudinal gradient. Staddon *et al*. (1998) reported a decrease in microbial diversity with increasing latitude resulting in an increased environmental harshness, decreased soil nutrient content and increased soil acidity. It was assumed that sufficient nutrients, conducive temperature, light intensity and soil pH (slightly acidic to neutral) at 300 m asl favored maximum cyanobacterial species diversity. In contrast, decrease in cyanobacterial species diversity at 1500 and 3500 m asl was associated with decreased nutrients and gradual increase in light intensity, extreme fluctuations in day-night temperature and soil acidity.

Differential selection of unicellular, non-heterocystous and heterocystous forms at different altitude is the function of increasing UV radiation, fluctuations in day-night temperatures and soil acidity in addition to nitrogen. It was evident from the study that in spite of low level of nitrogen at 3500 m asl, heterocystous (nitrogen-fixing) cyanobacterial species diversity was fewer in comparison to 1500 m asl with high level of nitrogen. Decrease in number of heterocystous cyanobacterial species and their sparse representation at higher altitude was assumed to be associated with increase for acidity and UV-B radiation with altitude (Bluthaler *et al.* 1992) which have negative effects on nitrogen fixation (Solheim *et al.* 2006). Moreover, adaptation to harsh conditions of high altitudes redirects the growth metabolic routes towards synthesis of UV-absorbing compounds and cryoprotectants thereby limiting the cyanobacterial proliferation. Some of them are carotenoid, astaxanthin, gloeocapsin, scytonemin and mycosporine (UV-B absorbing) (Bidigare *et al.* 1993) and high lipid, carbohydrate and glycine betaine (cryoprotectants) (Roser *et al.* 1992; Tearle 1987). The study concludes that cyanobacterial diversity along altitudinal gradient is the function of acidity, nitrogen and other climatic conditions.

Cyanobacteria flourish in diverse habitats with a remarkable ability to adapt in extreme conditions. The diversity of cyanobacteria in the Eastern Himalayan region of Arunachal Pradesh was found to be good. Heterocystous forms were more

diverse than non-heterocystous forms at moderate altitude of 1500 m asl; but at very high altitude of 3500 m asl, non-heterocystous forms became more prevalent. The change in community structure of cyanobacteria with increasing altitude emphasizes the need for an in-depth study for understanding the tolerance mechanisms along with the role of different stress proteins involved in the processes as well as for elucidating the metabolic machinery operating in such harsh conditions. The cyanobacterial community of high altitude regions may serve as a resource for their bioprospecting for cold stress proteins, UV-absorbing pigments and some antioxidant including astaxanthin and might provide some basic understanding of ecosystem functioning in natural ecosystem.

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References

Bidigare RR., Ondrusek ME, Kennicutt II MC, Iturriaga R., Harvey HR, Hoham RW & Mackov, S.A 1993 Evidence a photoprotective for secondary carotenoids of snow algae. J. Phycol. 29(4):427-434.

Blumthaler M, Ambach W & Rehwald W 1992 Solar UV-A and UV-B radiation fluxes at two alpine stations at different altitudes. Theor. Appl. Climatol. 46(1):39-44.

Choudhary KK 2009 Occurrence of Chrrococcaceae during rice cultivation in noerth Bihar, India. Bangladesh J. Plant Taxon. 16(1):57-63.

Choudhary K. 2011 Occurrence of nitrogen-fixing cyanobacterial during different stages of paddy cultivation. Bangladesh J. Plant Taxon. 18(1):73-76.

Desikachary TV 1959 Cyanophyta. Indian Council of Agricultural Research, New Delhi, India. pp. 1-686.

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seiber TM & Darzins AL 2008 Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54(4):621-639.

Kemprai R 2013 Biodiversity of Cyanobacteria in soils of Greater Haflong and their nitrogen fixing capacity. J. Algal Biomass Utln. 4(2):1-6.

Linington RG, Gonzalez J, Urena L-D, Romero LI, Ortega-Barria E & Gerwick WH 2007 Venturamides A and B: Antimalarial constituents of the Panamanian marine cyanobacterium *Oscillatoria* sp. J. Nat. Prod. 70(3):397-401.

Micheletti E, Colica G, Viti C, Tamagnini P & De Philippis R 2008 Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. J. Appl. Microbiol. 105(1):88-94.

Piper CS 1966 Soil and plant analyses. Inter Science Publ. Inc., New York.

Purvis A & Hector A 2000 Getting the measure of the biodiversity. Nature 405(6783):212-218.

Rippka R, Josette D, Waterbury JB, Herdman M & Stanier RY 1979 Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111(1)**:**1-61.

Roser DJ, Melick DR, Ling HU & Seppelt RD 1992 Polyol and sugar content of terrestrial plants from continental Antarctica. Antarctic Sci. 4(4):413-420.

Sanchez-Baracaldo P, Haynes PK & Blank CE2005. Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. Geobiology 3(3):145-165.

Solheim B, Zielke M, Bjerke JW & Rozema J 2006 Effects of enhanced UV-B radiation on nitrogen-fixation in arctic ecosystems. Plant Ecol. 182(1):109-118.

Staddon WJ, Trevors JT, Duchesne LC & Colombo CA 1998 Soil microbial diversity and community structure across a climatic gradient in western Canada. Biodiv. Conserv. 7(8):1081-1092.

Tearle PV 1987 Cryptogamic carbohydrate release and microbial response during spring freeze-thaw cycles in antarctic fellfield fines. Soil Biol. Biochem. 19(4):381-390.