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Relative Abundance of Cyanobacteria in Soil of Khasi Hills, Meghalaya and Determination of Antioxidant Potential of some Dominant Species

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Abstract: The relative abundance and antioxidant potential of some dominant cyanobacteria in the selected landuse type of Khasi Hills, Meghalaya were evaluated. A total of 105 species were isolated. Species of the genus belonging to *Oscillatoria* were with relative abundance of 41 to 75%. However, species occurring throughout the year with high relative abundance were *Synechococcus aeruginosus* (77-82%), followed *Oscillatoria limosa* (75%) and *Anabaena spiroides* (72%). The genus of *Oscillatoria* (*O. curviceps* and *O. limosa*) were with high phenolic (77.05 and 73.15µgGAE/ml respectively) and flavonoid contents (65.10 and 47.33µgGE/ml respectively). These can also scavenge DPPH radical and superoxide anion more efficiently than the other species. *Oscillatoria* species which was observed with high relative abundance in this part of the region could be used as a good source of antioxidant.

Keywords: Relative abundance, Antioxidant, Cyanobacteria, *Oscillatoria*, Khasi hills

Soil ecosystems formed the most important non-aqueous habitat for the terrestrial algae. Tropical India provides a favourable environment for the growth of cyanobacteria (Chellappa et al 2004, Geethu and Shamina 2021). Cyanobacteria are the oldest phototrophic microorganisms and play an important role as primary producers and pioneer organisms in soil crust (Budel et al 2016) and improve carbon and nitrogen contents, aggregation and stability of soil and also secretes the exopolysaccharides (Rossi et al 2018). The search for natural antioxidant has gained much interest due to the toxicity of synthetic antioxidant (Andrade et al 2019). Several studies have demonstrated that the amount of carotenoids contributed to the antioxidant capacity of microalgae (Takaichi 2011). Cyanobacteria are known to be a source of bioactive substances with many potential antioxidant compounds (Singh et al 2017, Morone et al 2019). Antioxidants are effective because they can donate their own electrons to Reactive Oxygen Species (ROS) and thereby neutralizing its adverse effects (Kohen and Nyska 2002). Thus the study was conducted in order to isolate the cyanobacteria with high relative abundance which can then be used as antioxidants.

MATERIAL AND METHODS

Collection of samples: Soil was collected from lands under shifting cultivation (1st year, 2nd year and 3rd year), agricultural farmlands (potato/rice field, potato/maize field), forests and plantation (Mawphlang Sacred Grove, pine forest and citrus plantation) from various locations of Khasi hills, Meghalaya. Surface soil and soil near the roots regions were collected randomly from 15 different parts of collection spots (10 m^2) with each collection spot approximately 10 m apart from each other. Soil samples from each collection spots were mixed together to form a composite mixture for the study of cyanobacteria community structure in selected land use type.

Preparation of samples: About 10 g of each soil sample was placed in a flask and diluted 100-fold with distilled water. BG 11 culture media was used in liquid or solidified form with 1.5% agar were used for culture and isolation. All the isolated cyanobacteria were observed under trinocular microscope (Olympus BX41) and identified to the possible lower taxonomic level by consulting standard books and monographs (Desikachary 1959, Philipose 1967, Prescott 1982, John et al 2002). Classification of cyanobacteria was carried out in accordance with Komárek et al (2014) and Guiry and Guiry (2020). The dominant cyanobacteria were grown in controlled condition of temperature at 25±1°C in light intensity of 40 µmol photons m^{-2} s^{-1} for 30 days. Culture experiments were conducted under a regime of 16 hour light/8 hour dark.

Data analysis: Relative abundance of a species was calculated (Dey et al 2010),

Relative abundance = Y/X 100

Where, X = total number of samples collected, Y = number of samples from which soil cyanobacteria was isolated

Preparation of cyanobacteria extract: 1g of air dried material was extracted at room temperature using methanol. It was then filtered through a filter paper (Whatman 1) and evaporated to dryness. The extract was then dissolved in methanol and store in the refrigerator at 20°C.

Analysis of antioxidant compounds and activity: The total phenolic content was determined by following Slinkard and Singleton (1977) using Folin & Ciocalteau reagent and gallic acid as standard whereas total flavonoid was determined by spectrophotometric method given by Quettier-Delue et al (2000). DPPH radical scavenging activity was measured by method described by Brand-Williams et al (1995). The superoxide anion radical scavenging activity was measured following the methods given by Nishikimi et al (1972). The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

RESULTS AND DISCUSSIONS

Distribution and relative abundance of cyanobacteria: Altogether 105 species belonging to 5 orders were observed in soils of different landuse type. Oscillatoriales (38%) were recorded to be dominant order followed by Nostocales, Synechococcales, Chroococcales and Spirulinales (Fig. 1).

Relative abundance was higher in agricultural farmlands followed by forests and plantation and lower in shifting cultivated land. *Synechococcus aeruginosus* was observed in soil of all landuse except in sacred grove. The highest relative abundance was in pine forest (82%) followed by citrus plantation.This could be attributed to the ability of this genus to tolerate stress (Stuart et al 2009). Some species observed with high abundance were *Anabaena spiroides* (72%) followed by *An. variabilis*,*An. oscillaroides* and *An. spiroides*. Some *Scytonema* species such as *Sc. hyalinum*, *Sc. geitleri*and *Sc. schmidtii* were observed in the form of crust (Table 1). Species of *Nostoc*, *Anabaena*, *Cylindrospermum* and *Scytonema* are widespread in Indian soil and were known to contribute abundantly to soil fertility (Nayak and Prasanna, 2007). *Aphanothece castagnei* and *Gloeothece rhodochlamys* were both observed in soils of potato/rice field with a higher abundance (66.6%). Species of *Pseudoanabaena batrachospermum* (52%) were observed in soil of potato/rice field. Species of *Oscillatoria* observed during rice cultivation in potato/rice field were *O. limosa* (75%) followed by *O. curviceps* (65%), *O. prombosidea* (45%), *O. princeps* (42%), *O. agardhii* (41%) (Table 1). Vijayan and Ray (2015) observed the dominance of Oscillatoriales revealed the ecological status of agroecosystem as a result of agricultural practices.

Antioxidant compounds and potential of dominant species: *O. curviceps* has the maximum amount of phenolic (77.05 µgGAE/ml) and flavonoid content (65.10 µgGE/ml). *Gloeothece rhodochlamys* was with lowest amount of phenolic (9.43 µgGE/ml). Kharkongor and Ramanujam (2017) recorded highest amount of phenolic compound could be the reason for a speciesto exhibit the highest DPPH radical and superoxide anion radical scavenging activity. It is already known that the smaller DPPH and Superoxide anion radical scavenging activity at % inhibition IC_{50}, the better is the ability of an algal species to scavenge these radical and anions. Thus *O. curviceps* (156.23 µg/ml) could scavenge DPPH radical more efficiently than *Aphanothece castagnei* (786.89µg/ml). The same could also be accounted for superoxide anion scavenging activity in which *O. curviceps* (213.54µg/ml)can scavenge superoxide anion more efficiently than *Synechococcus aeruginosus* (889.14µg/ml). The antioxidant properties of some selected cyanobacteriain which DPPH radical scavenging activity was highest in *Oscillatoria* sp that could be used as excellent source of anti-oxidant. Guerreiro et al (2020) observed that cyanobacteria from paddy fields have high levels of carotene. Therefore, antioxidant activity could possibly be attributed to the presence of high amount of carotenoids in *Oscillatoria* sp. Martinez and Barbosa (2008) concluded that carotenoids could quench radicals by hydrogen atom transfer or by accepting electrons from radicals. Kharkongor and Ramanujam (2017) observed highest amount of phenolic compound could be the reason for a species to exhibited highest DPPH radical scavenging activity and superoxide anion radical scavenging activity (Table 2).

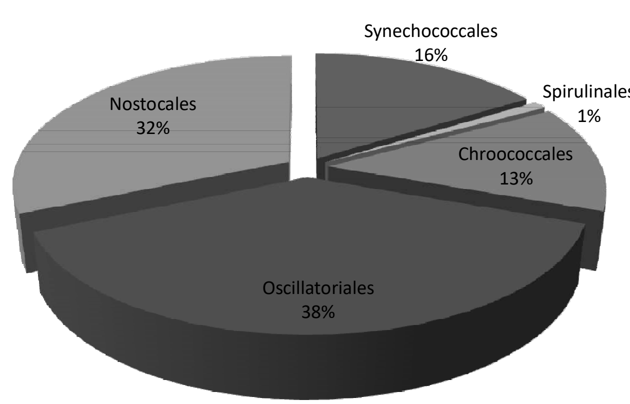

Fig. 1. Distributional pattern of different orders of Cyanobacteria

Table 1. Relative abundance and distribution of Cyanobacteria in soil of Khasi hills, Meghalaya

Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
Phylum - Cyanobacteria Class - Cyanophyceae								
Order - Synechococcales								
Aphanocapsa elachista West & G.S. West	-	-	+ *	-	-	+ *	-	-
A. incerta (Lemmermann) G. Cronberg & Komárek	-	-	-	+ *	-	-	-	-
Leptolyngbya battersii Gomont	-	-	-	-	+ *	-	-	-
L.faveolarum (Gomont) Anagnostidis & Komárek	-	-	+	-	-	-	-	-
L. vincentii Komárek	-	-	-	+ *	-	-	-	-
Merismopedia sp	-	-	-	-	-	-	-	+ *
Pseudoanabaena batrachospermorum (Skuja) Anagnostidis & Komárek	-	-	-	+ **	-	-	-	+ *
P. catenata Lauterborn	-	-	-	-	-	-	-	+
P. galeata Böcher	-	-	-	+ *	+ *	-	-	-
Pseudoanabaena sp	-	-	-	-	-	-	-	+ *
Schizothrix borealis Komárek & Kovacik	-	-	-	-	-	+ *	-	-
S. lateritia Kützing ex Gomont	-	-	-	-	-	+ *	-	-
S. telephoroides Gomont	-	-	-	-	-	-	+ *	-
S. tenuis Woronichin	-	-	-	-	-	-	-	+ *
S. tinctoria Gomont ex Gomont	-	-	-	-	-	-	+ *	-
Synechococcus aeruginosus Nägeli	+ *	+ *	+ *	+ *	+ *	-	+ ***	+ ***
*Sy. elongatus (*Nägeli) Nägeli	-	-	-	+ *	-	-	-	-
Order - Spirulinales								
Spirulina laxissima G.S. West	-	-	-	+ *	-	-	-	-
Order - Chroococcales								
Aphanothece castagnei (Kützing) Rabenhorst	-	-	-	+ ***	-	-	-	-
Ap. densa Silva	-	-	-	-	+ *	-	+ *	-
Chlorogloea novacekii Komárek & Montejano	-	-	-	-	-	+	-	+ **
Chlorogloea sp	-	-	-	-	-	-	-	+ *
Chroococcus minor (Kützing) Nägeli	-	-	-	+ **	-	-	-	+ **
Ch. tenax (Kirchner) Hieronymus	-	-	-	+ *	-	-	-	-
Gloecapsa decorticans (A. Braun) P. Richter	-	-	-	-	-	-	+ *	-
G. alpina Nägeli	-	-	-	-	-	+ *	-	-
G. muralis Kützing	-	-	-	+ **	-	-	-	-
G. rupestris Kützing	-	-	-	-	-	-	-	+
Gloeothece rhodochlamys Skuja	-	-	-	+ ***	-	-	-	-
Gl. tepidariorum (A. Braun) Lagerheim	-	-	-	-	-	+	-	-
Microcystis aeruginosa Kützing (Kützing)	-	-	-	-	+*	-	-	-
M. smithii Komárek & Anagnostidis	-	-	-	+	-	-	-	-
Order - Oscillatoriales								
Geitlerinema splendidum (Greville ex Gomont) Anagnostidis	+	-	-	+	-	-	-	-
Ge. unigranulatum Singh	-	-	-	+	-	-	-	-
Lyngbya dendrobia Brühl & Biswas	-	-	-	+ **	-	-	-	-
L. martensiana Meneghini ex Gomont	-	-	-	+ **	-	-	-	-
L. putealis Montagne ex Gomont	-	-	-	+ *	-	-	-	-
L. semiplena J. Agardh ex Gomont	-	-	-	-	-	+ *	-	-

Cont...

Table 1. Relative abundance and distribution of Cyanobacteria in soil of Khasi hills, Meghalaya

Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
L. shackletonic West & G. S. West	-	-	-	+ *	-	-	-	-
L. taylorii Drouet & Strickland	-	-	-	-	-	+	-	-
Lyngbya sp	-	-	-	-	-	-	+	-
Microcoleus chthonoplastes Thuret ex Gomont	-	-	-	-	-	+ *	-	-
Microcoleus sp	-	-	-	-	-	-	-	+ *
Oscillatoria agardhii Gomont	-	-	-	+ **	-	-	-	-
O. pseudogeminata G. Schmid	+	+ *	-	-	+	-	+	+ *
O. santa Kützing ex Gomont	-	-	-	-	+ *	-	-	-
O. tenuis C. Agardh ex Gomont	-	-	+ *	-	-	-	-	+ *
O. vizagapatensis Rao	-	-	-	+	-	-	-	-
O. curviceps C. Agardh ex Gomont	-	-	+ **	+ ***	+ **	-	+ *	-
O. irrigua Kützing ex Gomont	-	+ *	+ **	+ *	-	-	-	-
O. limnetica Lemmermann	-	-	-	-	-	-	-	+ **
O. limosa C. Agardh ex Gomont	-	-	-	+ ***	+ **	+ *	-	-
O. princeps Vaucher ex Gomont	-	-	-	+ **	-	-	-	-
O. tergestina Kützing	-	-	-	+	-	-	-	-
O. amphibia Agardh ex Gomont	-	-	-	+ *	-	-	-	-
O. anguina Bory ex Gomont	-	-	-	+ *	-	-	-	-
O. brevis Kützing ex Gomont	-	+ **	-	-	-	-	-	-
O. germinata Schwabe ex Gomont	-	-	-	-	-	+	-	+ ***
O. nigra Vaucher ex Gomont	-	-	-	-	-	+	-	-
O. proboscidea Gomont	-	+ *	-	+ **	-	-	-	-
O. rubescens De Candolle ex Gomont	-	-	-	+	-	-	-	+
Oscillatoria sp	-	-	-	+ *	-	-	-	-
Phormidium abronema Skuja	-	-	-	+ *	+ *	-	-	-
P. inundatum Kützing ex Gomont	-	-	-	-	-	-	+ *	-
P. papyraceum Skuja	-	-	-	+ **	-	-	-	-
P. retzii Kützing ex Gomont	-	-	-	-	-	-	+	-
P. tenue Gomont	+	+ *		-	+ **	-	-	-
P. corium Gomont ex Gomont	-	-	-	-	-	-	-	+
Phormidium sp 1	-	-	+	-	-	-	-	-
Phormidium sp 2	-	-	-	+	-	-	-	-
Plectonema sp	-	-	-	-	-	-	-	+
Symploca muscorum Gomont ex Gomont	-	-	-	-	-	+	-	-
Order - Nostocales								
Amorphonostoc pruniforme (Kützing) Elenkin	-	-	-	+	-	-	-	-
Anabaena constricta (Szafer) Geitler	-	-	-	-	-	-	+	-
An. catenula Kützing ex Bornet & Flahault	-	-	-	-	-	+	+	-
An. iyengarii Kharadwaja	-	-	-	+ **	-	-	-	-
An. oryzae F. E. Fritsch	-	-	-	+ **	-	-	-	-

Cont...

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Taxa	Shifting cultivation			Farmlands		Forests and plantation		
	SC1	SC2	SC3	P/R	P/M	MSG	PF	CP
An. oscillarioides (Bory ex Bornet) Flahault	-	-	+ **	-	-	+ **	-	-
An. sphearica Bornet & Flahault	-	-	-	+ **	-	-	-	-
An. spiroides Klebahn	-	-	+ **	-	+ **	-	+ ***	+ ***
An. torulosa Lagerheim ex Bornet & Flahault	-	-	-	+	-	-	-	-
An. variabilis Kützing ex Bornet & Flahault	-	-	-	+ *	+ **	-	-	+***
Aulosira sp	-	-	-	+ **	-	-	-	-
Calothrix sp 1	-	-	-	+ *	-	-	-	-
Calothrix sp 2	-	-	-	-	-	-	-	+ *
Cylindrospermum michailovskoense Elenkin	-	-		-	-	+ *	-	-
Cy. muscicola Kützing ex Bornet & Flahault	-	-	-	-	-	+ *	-	-
Nostoc carneum C.Agardh ex Bornet & Flahault	-	-	-	+ **	-	+ **	-	+ *
N. commune Vaucher ex Bornet & Flahault	-	-	-	-	-	+*	-	-
N. linckia Bornet ex Bornet & Flahault	-	-	-	-	-	-	+ *	-
N. muscorum C. Agardh ex Bornet & Flahault	-	-	-	-	+ **	-	-	-
N. padulosum Kützing ex Bornet	-	-	-	+*	-	-	-	-
N. pruniforme Hariot	-	-	-	-	-	-	+ *	-
N. spongiaeformae C. Agardh ex Bornet & Flahaut	-	-	-	+ **	-	-	-	-
Scytonema geitleri Bharadwaja	-	-	-	-	-	-	-	+ **
Sc. hyalinum Gardner	-	-	-	-	-	-	-	+ **
Sc. mirabile Bornet	-	-	-	-	-	+ *	-	-
Sc. schmiditii Gomont	-	-	-	-	-	-	-	+ **
Scytonema sp 1	-	-	-	+ **	-	-	-	-
Scytonema sp 2	-	-	-	-	-	+ *	-	-
Stigonema hormoides Bornet & Flahault	-	-	-	-	-	-	-	+ **
St. mammilosum Agardh ex Gomont	-	-	-	-	-	-	+ **	-
St. ocellatum Thuret ex Bornet & Flahault	-	-	-	-	-	+ *	-	-
Tolypothrix sp	-	-	-	-	-	-	-	+ *
Westiellopsis sp	-	-	-	-	-	+ *	-	-

(SC 1 - 1st year, SC 2 - 2nd year, SC 3 - 3rd year, P/R - Potato/Rice field, P/M - Potato/Maize field, MSG - Mawphlang Sacred Grove, PF - Pine Forest, CP - Citrus Plantation). Relative abundance - (*) indicates 20% to 39%, (**) indicates 40% to 59%, (***) indicates > 60%; ' + ' indicates present, ' - ' indicates absent)

Table 2. Antioxidant potential of some dominant cyanobacterial species

Cyanobacteria species	Total phenolics content (μgGAE/ml of extract)	Flavonoid content (μgGE/ml of extract)	DPPH Radical scavenging activity IC_{50} (μg/ml)	Superoxide anion scavenging activity IC_{50} (μg/ml)
Anabaena oscillarioides	23.12±1.67	12.34±0.98	456.34±1.54	543.78±0.34
An. spiroides	24.00±2.12	11.23±1.65	567.56±0.45	678.10±1.08
An. variabilis	24.12±1.87	11.19±1.34	456.21±1.12	657.05±1.87
Aphanothece castagnei	25.26±1.19	15.23±2.34	786.89±4.23	345.54±3.09
Gloeothece rhodochlamys	27.56±1.21	12.43±1.45	689.19±5.67	452.16±8.12
Oscillatoria curviceps	77.05±1.23	65.10±1.89	156.23±9.54	213.54±4.12
O. germinata	54.10±1.45	48.34±3.23	167.65±7.23	256.19±1.23
O. limosa	73.15±3.56	47.33±5.34	172.29±10.21	237.45±9.65
Scytonema geitleri	57.34±5.78	42.76±1.42	598.21±2.65	256.87±1.87
Sc. hyalinum	40.17±2.22	19.45±1.98	456.34±2.98	548.21±1.98
Sc. schmidtii	41.86±3.89	21.24±2.67	324.56±12.09	439.10±8.43
Synechococcus aeruginosus	21.16±4.32	10.28±1.88	764.17±3.12	889.14±1.87

Values are mean ± SE of three replicates

CONCLUSION

All the twelve cyanobacterial species have some antioxidant activity. *O. curviceps* has the maximum amount of phenolic and flavonoid content. This species also scavenged DPPH radical and superoxide anion more efficiently than all the other species. Other species with potentially higher scavenging activity was observed for *O. germinata*, O. *limosa* and *Scytonema geitleri*. The high antioxidant activity along with high relative abundance of these species can be an excellent source of raw materials from this part of the region.

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