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MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CYANOBACTERIAL ISOLATES FROM BIOLOGICAL SOIL CRUST OF RAJASTHAN, INDIA

Lokesh Sharma, Shailendra Kumar Singh, Shruti Srivastava and Shanthy Sundaram *

Centers of Biotechnology, University of Allahabad, Prayagraj - 211002, Uttar Pradesh, India.

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Correspondence to Author:
Shanthy Sundaram

Professor and Coordinator, Centers of Biotechnology, University of Allahabad, Prayagraj - 211002, Uttar Pradesh, India.

E-mail: shanthy.cbt@gmail.com

ABSTRACT: The BSC sample was collected at four sites from the arid region of Rajasthan and these isolates were characterized based on morphological and biochemical parameters. We have to isolate both heterocysts and non-heterocysts cyanobacterial species. *Oscillatoria tenuis* species showed maximum chlorophyll accumulation and Phormium showed maximum carotenoids compared to heterocystous forms. The soluble protein content is high in Non-heterocystous Phormium and *Oscillatora curviceps* and lowest in heterocystous *Nostoc linckia*. Heterocystous forms Nostoc commune and *Anabaena variabilis* were synthesizing high carbohydrates compared to nonheterocytstous forms. Highest nitrogenase activity in *Nostoc linckia* isolated from Achorl site.

INTRODUCTION: Cyanobacteria is group of primitive phototrophic prokaryotic organisms whose long evolutionary history dates back to the Proterozoic era. These organisms, endowed with tremendous genome plasticity, are distributed in all possible biotypes of the world. Due to their occurrence in diverse habitats, these organisms are the excellent material for investigation by ecologists, physiologists, biochemists and molecular biologists. These organisms also have tremendous potential in environmental management as soil conditioners, biofertilizers bio monitors of soil fertility and water quality, amelioratory agents in reclamation of saline and usar lands, and in rehabilitation of degraded ecosystems through biosorption of metals, feed for animals and protein supplement [1].

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Distribution of these organisms in diverse habitats has always attracted attention of scientists for evolving suitable methods for their ecological investigations. Cyanobacteria dominate the micro-algal populations of hot deserts and comprise a vast majority of micro-flora in arid zones, both in soil crusts [2] and endo-lithic communities. In hot arid zones, these are exposed to more sudden environmental changes because of daily transitions from hot and dry conditions to warm and humid ones when fog and dew may condense at night [3, 4]. Cyanobacteria have a remarkable suite of attributes and strategies, which enable them to colonize and survive under extreme habitats. These organisms are able to interact with their niche, develop certain survival mechanisms and either exploit or modify their attributes to make them more suitable under water stress conditions [5, 6].

A host of factors that affect the distribution of cyanobacteria include pH, soil moisture, mineral nutrients, CO_2 fixation and combined nitrogen [7, 8, 9]. Due to the pivotal role played by these organisms, it was considered worthwhile to examine the

existence of cyanobacteria in arid zones of Rajasthan, India, and to analyse their growth parameters and physiological attributes for possible biotechnological applications.

MATERIALS & METHODS:

Soil Sample Collection: BSC sample collected in four sites from Rajasthan. Soil spots randomly selected and scraping about 200 g soil from upper 1cm soil layer from 0.5 ha area had well-developed biocrusts, representing a range of colonization states, from early successional (lightly pigmented: light biocrusts) to late successional (darkly pigmented: dark biocrusts) [10, 11].

Biocrust at each site were collected **Fig. 1**, using Petri dishes (90 mm diameter, 15 mm deep), allowed to dry completely, sealed in zippered plastic bags, and stored in the dark at room temperature until use [12].

FIG. 1: MICROGRAPHS SITE OF BSC SAMPLE COLLECTION (A) ACHROL (B) VIRATNAGAR (C) ALWAR (D) KUSALGARDH

Cyanobacterial Culture: The enrichment culture technique was utilized for deciphering cyanobacterial populations in the arid zones [13, 14, 15]. For the isolation of strains from representative spots, 1 g soil sample was inoculated in 50 mL sterilized BG–11 medium in the absence and presence of nitrogen (1.5 g $NaNO_3$ L–1). Flasks were incubated for 30 days at 28 ± 20C and 3–4 K lux light intensity with cool white fluorescent light tubes under 16/8 h light and dark cycles (Stanier *et al.* 1971). The cyanobacterial strains were isolated by Serial dilution and pour plate method.

The taxonomic identification was done following the keys given by Desikachary (1959) and Starmach (1966) [8]. The non-heterocystous isolates are maintained in nitrogen enriched (+N), and heterocystous isolates are maintained in a nitrogen deficient (-N) medium in a temperature-regulated culture room under controlled light and dark cycles.

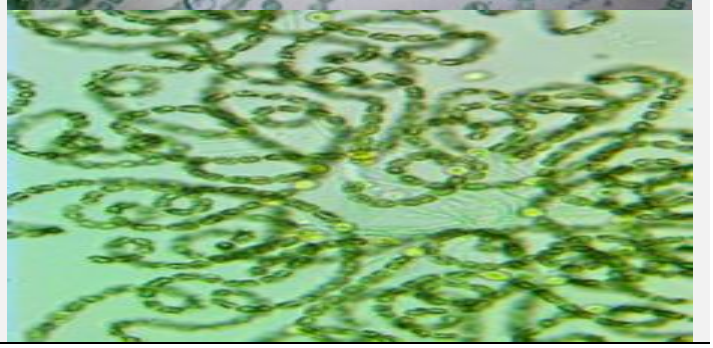
Photosynthetic Pigments Analysis: A cyanobacterial culture was centrifuged and the pellet was suspended in acetone to determine photosynthetic pigments (80 percent) at 4ºC. The cells were incubated overnight. The sample was centrifuged again for 5 minutes at 10,000 rpm [22] and the absorbance of the supernatant for chlorophyll and carotenoids was measured at 665 and 480 nm, respectively, using UV–Vis spectrophotometer (Pharmacia Biotech). The quantity of Chl a was determined using [23] formula, and the total amount of carotenoids was computed using the specific formula Jensen's absorption coefficient as described in 1978. The absorbance of the blue supernatant generated in phosphate buffer was measured using a cyanobacterial pellet and repeated freezing and thawing. (pH of 7.5) at 620 nm and the phycocyanin content was computed in accordance with [24, 25].

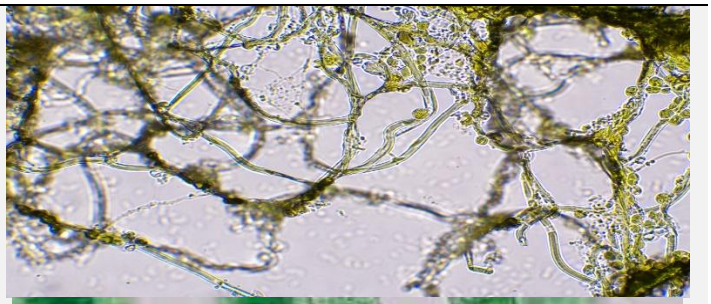
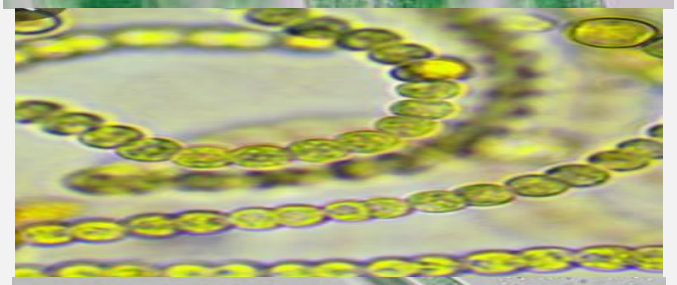
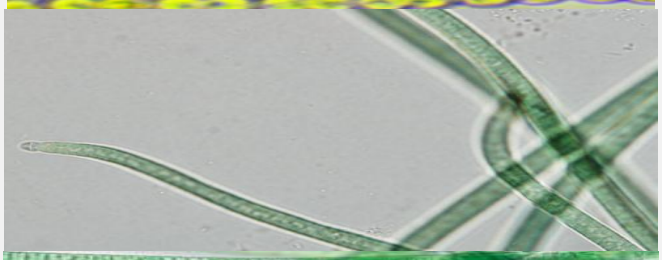
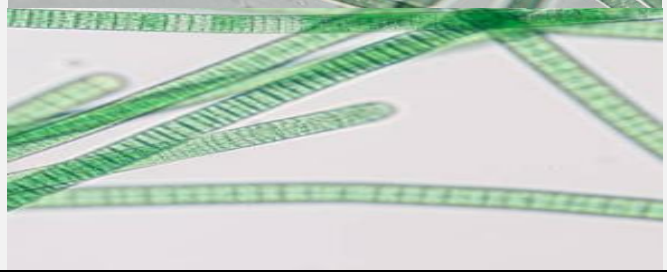
Metabolite Content & Nitrogen Fixation Ability: Under controlled circumstances, cultures were grown in nitrogen deficient medium for heterocystous, nitrogen fixing forms, and nitrogen enriched media for non-heterocystous forms to determine chlorophyll accumulation, metabolite content, and nitrogen fixation capacity. All tests were carried out three times. Samples were fully homogenised using a shaker or homogenizer and collected for further examination during the exponential phase of development (15 days incubation). The content of chlorophyll was determined using the hot methanol extraction technique [26, 27].

Total carbohydrates were determined using the anthrone-thiourea technique [28] and soluble proteins were calculated using bovine serum albumin as a reference [29, 30, 31]. Solarzano's apprach was used to investigate extracellular ammonia emission. The acetylene reduction test [32, 33] was used to measure nitrogenase activity using a gas chromatograph (Nucon Model GC 5700) with a Porapak N column. Nitrogenase activity was measured using ethylene generated in the gas phase, and the activity was represented as n mole C_2H_4 mg^{-1} chl h^{-1}[34].

RESULTS & DISCUSSION: Morphological characters were identified microscopically based upon the keys given by Desikachary (1959) **Table 1.** Studies undertaken clearly indicated ubiquitous occurrence of cyanobacteria in water stress habitats. Results showed that Anabaena &Nostoc was predominantly present in the arid areas surveyed. A wide variation was observed among different cyanobacterial strains isolated from arid zones of Rajasthan, India, with respect to chlorophyll accumulation, carotenoids, carbohydrate, soluble protein content and the nitrogen fixing potential **Table 2** and **Table 3.**

TABLE 1: MICROSCOPY IMAGE OF ISOLATED SPP. AND NAME OF SITE

Site name	Isolated spp.	Microscopy image
Achrol (Site1)	*Anabaena sphaerica*	
	Nostoc linckia	
Viratnagar Site 2	*Anabaena variabilis*	

	Nostoc verrucosm	
Site-3-Alwar	*Oscillatoracurviceps*	
	Nostoc commune	
Site-4 Kusalgardh	*Phormidium*	
	Oscillatoria tenuis	

Chlorophyll content varied in isolated spp. from Achrol, Viratnagar, Alwar and Kushalgarh. Two isolates from *Anabaena sphaerica* show high chlrophyll content (7.2 ug mL^{-1}) then *Nostoc linckia* is low (1.3 µg mL^{-1}) but nearly same carotenoid content isolated from the soils of Achrol. *Anabaena variabilis* isolated from arid zones of Viratnagar, showed highest chlorophyll content (5.4 µg mL^{-1}) whereas *Nostoc verrucosum* accumulated lowest chlorophyll content (1.1 µg mL^{-1}) and carotenoids content is 4.4 to 3.4 µg mL^{-1}. Isolates from Alwar, *Oscillatora curviceps* showed highest chlorophyll accumulation (7.3 µg mL^{-1}) indicating it to be a slow growing form and *Nostoc commune* accumulated chlorophyll very less (1.5 µg mL^{-1}) and carotenoid content 5.3 µg mL^{-1} to 0.3 µg mL^{-1} respectively. In Kushalgarh, *Phormidium* and *Oscillatoria tenuis* show 7.5 µg mL^{-1} to 10.3 µg mL^{-1} chlorophyll and carotenoid is 12.3 µg mL^{-1} to 5.6µg mL^{-1} respectively.

Out of various isolates, the chlorophyll accumulation during exponential phase of growth was highest in Oscillatoria tenuis (10.3 µg mL^{-1}) followed by *Phormidium, Oscillatora curviceps* and *Anabaena sphaerica* nearly 7 µg mL^{-1}.

Interestingly, another species *Phormidium* exhibited highest carotenoid accumulation (12.3 µg mL^{-1}) and lowest in *Nostoc commune* 0.3 µg mL^{-1}.

Soluble Protein & Carbohydrate Content: *Anabaena sphaerica*, an isolate from Achrol, produced highest soluble proteins (357.2 µg mL^{-1}) and *Nostoc linckia* produced lowest soluble protein (77.3 µg mL^{-1}). In Viratnagar *Anabaena variabilis* highest 247.2 µg mL^{-1} and lowest in *Nostoc verrucosu*m 82 µg mL^{-1}. The non–hetrocystous strain, *Oscillatora curviceps* isolated from the Alwar soil exhibited maximum soluble protein content (432.5 µg mL^{-1}), followed by heterocystous strain, *Nostoc commune* (115.0 µg mL^{-1}). Soluble proteins by the isolates of Kushalgarh was markedly high than the ability observed by the strains isolated from Achrol , Viratnagar and Alwar. Out of all isolates from different sites soluble protein content is lowest in *Nostoc linckia&Nostoc verrucosum* 77.3 µg mL^{-1} to 82 µg mL^{-1} respectively and highest in non-heterocyst *Phormidium , Oscillatora curviceps* and *Oscillatoria tenuis* 544.1 µg mL^{-1},432.5 µg mL^{-1} to 421.3µg mL^{-1} respectively Carbohydrates content (µg mL^{-1}) examined form different in different arid zones of Rajasthan also showed a variable pattern. *Anabaena sphaerica* isolated from the soils of Achrol showed maximum carbohydrate (22.2 µg mL^{-1}), followed by the carbohydrate content shown by *Nostoc linkia* (7.5 µg mL^{-1}).

Anabaena variabilis isolated from the soils of Viratnagar showed highest carbohydrate content (129.5 µg mL^{-1}) and low in Nostoc verrucosum is 12.3ug mL^{-1}. Isolated from the solids of Alwar showed that the carbohydrate contents was low in non-hetrocysts *Oscillatora curviceps* and high in *Nostoc commune* that is 102.5 µg mL^{-1}. In Kusalgardh the carbohydrate content was lowest among all the isolates in non-heterocyst *Phormidium* and *Oscillatoria tenuis* 2.2 µg mL^{-1} to 4.4 µg mL^{-1} respectively.

TABLE 2: CHARACTERIZATION OF CYANOBACTERIAL STRAINS ISOLATED FROM ARID ZONES OF RAJASTHAN, INDIA

Place/ Name of Isolates	Chlorophyll (µg mL–1) /	Carotenoid (ug mL-1)	Soluble Proteins (µgmL1)	Carbohydrates (µg mL–1)
Site-1		**2.3**	**497.8**	**22.2**
Anabaena sphaerica	7.2	2.3	357.2	22.2
Nostoc linckia	1.3	2.5	77.3	7.5
Site-2				
Anabaena variabilis	5.4	4.4	247.2	129.5
Nostoc verrucosm	1.1	3.4	82	12.3
Site-3				
Oscillatoracurviceps	7.3	5.3	432.5	4.2
Nostoc Commune	1.5	0.3	115.0	102.5
Site-4				
Phormium	7.5	12.3	544.1	2.2
Oscillatoria tenuis	10.3	5.6	421.3	4.4

*Being non-heterocystous forms not exhibiting nitrogenase activity, NS = nonsignificant

Enzymatic Activity & Extracellular Ammonia Release: In terms of nitrogenase activity, *Nostoc linckia* (1202.7 n mole C_2H_4 mg^{-1} chl h^{-1}) was the most efficient strain from Achrol, whereas *Anabaena sphaerica* (173.0 n mole C_2H_4 mg^{-1} chl h^{-1}) was the least efficient. In Viratnagar *Nostoc varrucosum* had the highest nitrogenase activity 750 n mole C_2H_4 mg^{-1} chl h^{-1}), whereas Anabaena variabilis had the lowest (85.1 n mole C_2H_4 mg^{-1} chl h^{-1}). Isolates from Alwar *Nostoc commune* had 435.2 n mole C_2H_4 mg^{-1} chl h^{-} nitrogenase activity.

TABLE 3: ENZYMATIC ACTIVITY OF CYANOBACTERIAL STRAINS

Place/ Name of Isolates	Nitrogenase Activity (n mole C2H4 mg-1 chl h–1)	Extracellular Ammonia Release (µmole NH4+ mL–1)
Site-1	**163**	
Anabaena sphaerica	173.0	0.004
Nostoc linckia	1202.7	0.003
Site-2		

Anabaena variabilis	85.1	0.03
Nostoc verrucosm	750	0.01
Site-3		
Oscillatoracurviceps	*	0.01
Nostoc commune	435.2	0.003
Site-4		
Phormidium	*	0.02
Oscillatoria tenuis	*	0.04

Under aerobic circumstances, *Phormidium, Oscillatoria curviceps* & *Oscillatoria tenuis* showed any nitrogenase activity due to their non heterocystous nature. The Strains isolated from soils of Achrol, Viratnagar, Alwar and Kushalgarh showed negligible amount of ammonia release.

The maximum extracellular ammonia release was only 0.04 µmole NH_4^+ mL^{-1}) by *Oscillatoria tenuis* from Kushalgarh, an isolate from Achrol showed lowest extracellular ammonia release of 0.003 & 0.004 in *Nostoc linckia* and *Anabena sphaerica* respectively.

CONCLUSION: In present work we evaluate morphological and biochemical diversity of cyanobacterial species in arid region of Rajasthan. Presence of photosynthetic pigments, biomolecules and nitrogenase enzyme ability shows that biological soil crust have cyanobacterial species and help in soil productivity in arid region of Rajasthan.

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