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SHORT COMMUNICATION

DISTRIBUTION AND CHARACTERIZATION OF SELECTED RIVULARIACEAE MEMBERS OF CYANOBACTERIA, ORDER NOSTOCALES

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Cyanobacterial strains from family Rivulariaceae were isolated from various localities of District Allahabad, UP. Variations were observed with respect to photosynthetic rate, respiratory activity, carbohydrates and total soluble proteins amongst strains of *Calothrix*, *Dichothrix* and *Gloeotrichia*. The total N content and nitrogenase activity also varied in the three genera examined. The studies indicated the dominance of cyanobacteria belonging to family Rivulariaceae in the rice field soils and the diversity existed among these with respect to physiological parameters.

***Key words*: Nitrogenase activity, photosynthesis, respiration, Rivulariaceae.**

Cyanobacteria are morphologically diverse group of prokaryotes inhabiting wide range of soils, both on and below the surface (De 1939). The frequent occurrence of cyanobacteria on the surface of tropical soils could be attributed to the optimum temperature under these conditions especially in wetland rice fields (Ladha and Reddy 1995). The first cyanobacterium *Calothrix indica,* which is a member of family Rivulariaceae was described from Assam. The key characteristic of the family is trichome polarity, as evidenced by tapering at one end of the filament and polar (basal) heterocyst under appropriate environmental condition. Based upon the morphological attributes and cultural behaviour, a large number of traditional genera have been included in this family. *Calothrix* and to lesser extent *Gloeotrichia* and *Dichothrix* have been studied extensively (Desikachary 1959). Tropical soils of India and particularly that of flooded rice fields often have a diverse flora of morphologically distinct forms (Tiwari 1972, Venkataraman 1975, Saradeshpande and Goyal 1981, Sinha and Mukherjee 1984). The present study was undertaken to isolate, purify, identify and characterize the distinctive cyanobacterial members of family Rivulariaceae, order Nostocales, from soils of various localities of Allahabad, an important rice producing area of eastern UP in India.

Soil samples collected from thirty different locations of paddy fields and adjoining areas of district Allahabad, Uttar Pradesh, India were subjected to enrichment culture techniques for isolation of cyanobacteria belonging to family Rivulariaceae. The taxonomic identification was done following the keys given by Desikachary (1959) and Anagnostidis and Komarek (1985). The isolates were maintained and grown in BG 11 medium (Stanier *et al.* 1971) at 28 ± 2^0 C under cool white fluorescent light and 14 - 10 L/D cycle. The exponentially growing cultures were examined for photosynthetic and respiration rates, total soluble proteins (Lowry *et al.* 1951), carbohydrates (Spiro 1966), nitrogen fixing capacity in terms of nitrogenase activity (Hardy *et al.* 1973) and total nitrogen content (Humphries 1954). The rate of photosynthetic oxygen evolution and respiratory oxygen uptake was measured following Sestak *et al.* (1971). The treatments were replicated three times in each experiment and the data obtained

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subjected to statistical analysis using DMRT (Duncan's Multiple Range Test).

Cyanobacterial strains, representing seven species of *Calothrix*, two species of *Gloeotrichia* and one species of *Dichothrix* belonging to the family Rivulariaceae, were isolated from soil samples collected from district Allahabad. These were identified based upon visual observations and microscopic examination at different stages of their life cycles (Table 1). The occurrence, succession and establishment of cyanobacterial strains are known to be influenced by crop growth phases and management practices (Roger and Kulasooriya 1980). Rice fields and other cultivable habitats are known to provide an ideal environment for the growth and proliferation of cyanobacteria with abundance of members of Rivulariaceae (Kobayashi *et al*. 1967, Junoto 1973, Yoshida *et al*. 1973, Venkataraman 1979, Tripathi 2001). A wide variation was observed amongst and within the strains of *Calothrix*, *Dichothrix* and *Gloeotrichia* with respect to photosynthetic and respiratory rates, total soluble proteins, carbohydrate accumulation and nitrogen fixing activity (Table 2). While the photosynthetic rate and respiratory activity was highest in *Gloeotrichia raciborskii*, the lowest photosynthetic rate was exhibited by *Calothrix membranacea* and the respiratory activity was minimum in *Gloeotrichia* sp.. As per DMRT ranking *Calothrix membranacea*, *Dichothrix baueriana* and *Gloeotrichia raciborskii* produced highest carbohydrates whereas *Calothrix marchica* var. *crassa* produced highest total soluble proteins. However, the carbohydrates were lowest in *Calothrix braunii* and the total soluble proteins accumulated were lowest in *Dichothrix baueriana*. Interestingly, it is worthwhile to mention that the *Calothrix membranacea*, an isolate from Lalgopalganj, Allahabad which showed highest carbohydrate content (214.54 µg/ml) displayed a lowest photosynthetic rate (122.74 µmol O_2 evolved / mg chl. / h). Its respiration rate was also comparatively high and ranked second (Table 2). The results clearly suggested that the biomass produced and metabolites accumulated are under complex physiological processes, influenced by the environmental parameters and genetic structure of the organisms.

Gloeotrichia raciborskii showed highest nitrogenase activity followed by *Calothrix braunii*, *Calothrix fusca* var. *crassa* and *Gloeotrichia* sp. which had almost similar nitrogenase activity. Two isolates namely *Calothrix elenkinii* and *Calothrix membranacea* exhibited least nitrogenase activity. Total nitrogen content was highest in *Calothrix marchica* var.

Table 1. Morphological characterization of selected cyanobacterial strains from family Rivulariaceae.

Cyanobacterial strains / Area of isolation	Filament size (µm)						
	Filament width (µm)	Cell width			Cell length		
		Base	Mid	Tip	Base	Mid	Tip
Calothrix braunii (Phulpur, Allahabad)	16.0	15.0	12.5	5.0	8.0	5.0	9.0
Calothrix marchica var. *crassa* (Amreha, Allahabad)	7.0	6.25	5.0	1.25	6.25	7.5	5.0
Calothrix elenkinii (Phulpur, Allahabad)	6.0	5.0	4.5	4.0	2.0	2.5	3.5
Calothrix membranacea (Lalgopalganj, Allahabad)	8.0	7.5	6.0	4.5	8.0	8.0	7.5
Calothrix sp. (Naini, Allahabad)	7.0	6.0	4.0	2.5	5.0	4.0	5.0
Calothrix javanica var. *fertilissima* (Bara, Allahabad)	6.0	6.0	5.0	2.5	7.5	9.0	9.0
Calothrix fusca var. *crassa* (Amreha, Allahabad)	17.0	14.0	7.0	2.0	7.0	7.0	10.0
Dichothrix baueriana (Jhunsi, Allahabad)	19.0	15.0	12.0	5.0	4.0	8.0	4.0
Gloeotrichia sp. (Lalgopalganj, Allahabad)	14.0	9.0	7.0	5.5	15.0	7.0	5.0
Gloeotrichia raciborskii (Bara, Allahabad)	17.0	10.0	5.0	2.0	5.0	6.0	6.0

Table 2: Physiological parameters of selected cyanobacterial strains from family Rivulariaceae.

Genera	Photosynthetic rate (µmol O_2 evol. mg^{-1} chl h^{-1})	Respiratory rate (µmol O_2 abs. mg^{-1} chl h^{-1})	Carbohydrates (µg ml^{-1})	Total Soluble Proteins (µg ml^{-1})	Nitrogenase activity (nmol C_2H_4 μg^{-1} chl h^{-1})	Total Nitrogen (µg ml^{-1})
Calothrix braunii	225.01 [e]	23.64 [f]	99.16 [g]	68.60 [g]	3.98 [b]	9.98 [cd]
Calothrix marchica var. *crassa*	368.34 [b]	47.10 [c]	173.19 [c]	146.29 [a]	1.49 [cd]	18.99 [a]
Calothrix elenkinii	282.84 [d]	32.61 [e]	164.85 [d]	139.69 [b]	1.13 [d]	18.48 [a]
Calothrix membranacea	122.74 [h]	65.52 [b]	214.54 [a]	98.35 [c]	1.01 [d]	15.33 [abc]
Calothrix sp.	174.34 [g]	35.50 [de]	187.05 [b]	85.68 [d]	1.21 [cd]	13.50 [abcd]
Calothrix javanica var. *fertilissima*	199.84 [f]	40.77 [d]	133.52 [f]	79.62 [e]	1.63 [cd]	12.03 [bcd]
Calothrix fusca var. *crassa*	301.63 [c]	67.73 [b]	159.55 [e]	58.33 [h]	3.61 [b]	9.20 [cd]
Dichothrix baueriana	188.55 [fg]	35.02 [de]	215.56 [a]	51.38 [i]	1.97 [c]	7.01 [d]
Gloeotrichia sp.	227.14 [e]	15.61 [g]	171.84 [c]	136.58 [b]	4.16 [b]	18.23 [ab]
Gloeotrichia raciborskii	668.59 [a]	98.68 [a]	214.09 [a]	74.53 [f]	10.76 [a]	10.00 [cd]
CD at 5 % p	5.80	2.03	1.71	1.36	0.25	1.98

crassa and *Calothrix elenkinii* but *Dichothrix baueriana* showed the lowest nitrogen content. It was interesting to note that nitrogenase activity exhibited by the strains examined did not correlate with the total nitrogen content indicating thereby, the complexity of the interactions operating under intricate, heterogenous environment. The variability observed amongst strains analysed was in accordance with earlier studies (Marcelle 1975, Garcia - Pichel and Castenholz 1991, Dhar *et al.* 2000, Mishra *et al.* 2001a, 2001b).

The results exhibited immense variability among isolates with respect to different metabolic parameters. Photosynthetic and respiratory processes are interrelated, interdependent and are influenced by a variety of environmental/ biological factors (Heath 1969, Marcelle 1975). Similarly, both intra and intergeneric variations in the metabolic quantity and quality among cyanobacteria have been reported (Gerwick *et al*. 2001, Nagle and Paul 1999). The investigation clearly indicated the dominance of cyanobacteria belonging to family Rivulariaceae in the rice field soils and the diversity existed among these with respect to physiological parameters.

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