Indian J. Plant Physiol., Vol. 12, No. 2, (N.S.) pp. 181-185 (April-June, 2007)

SHORT COMMUNICATION

PHYSIOLOGICAL CHARACTERIZATION OF CYANOBACTERIAL ISOLATES FROM ORISSA AND WEST BENGAL

SUDHIR SAXENA, B.V. SINGH, SAURABH TIWARI AND DOLLY WATTAL DHAR*

Centre for Conservation and Utilisation of Blue Green Algae, Indian Agricultural Research Institute, New Delhi - 110 012

Received on 22 April, 2006, Revised on 25 Jan., 2007

A total of 34 cyanobacterial isolates from soils of Orissa and West Bengal were purified and identified based upon morphological parameters and these were submitted in the germplasm of Centre for Conservation and Utilisation of Blue Green Algae, Indian Agricultural Research Institute, New Delhi. Physiological characterization exhibited distinct variability amongst these isolates with respect to growth (dry weight), pigments, total soluble proteins, carbohydrates and nitrogen fixing potential.

***Key words*: Cyanobacteria, growth, pigments, proteins, carbohydrates, nitrogen fixation**

Cyanobacteria/blue green algae occupy a unique position, as they possess an autotrophic mode of growth like eukaryotic plant cells and a metabolic system as that of bacteria. These are gram negative, carry out oxygenic photosynthesis and possess chlorophyll *a*. They are characterized by a great morphological diversity and their widespread distribution reflects a broad spectrum of physiological properties and tolerance to environmental stress (Tandeau de Marsac and Houward 1993).

Detailed studies conducted on the distribution and periodicity of blue green algae from various parts of India have yielded interesting results (Venkataraman 1975, Kolte and Goyal 1985, Singh 1985). A number of reports have indicated widespread distribution of forms like *Oscillatoria*, *Nostoc*, *Anabaena*, *Phormidium* and *Aphanothece* (Gupta 1975, Sinha and Mukherjee 1975, Paul and Santra 1982). The present study analysed the cyanobacterial isolates from soils of Orissa and West Bengal for physiological attributes.

Soil samples were collected from different locations of Bhubaneshwar, Cuttack and Howrah by removing surface debris of 4-5 randomly selected spots. From each spot, 100 g soil from upper 1cm layer was lifted, thoroughly mixed, sun dried (25-35^{0}C, RH 30-60%) and sieved. Out of these, representative sample of 200 g was stored in sample bottles.

For the isolation of cyanobacterial strains, 1 g soil was inoculated in 50 ml sterilized BG-11 medium in the presence (1.5 g l^{-1} $NaNO_3$) and absence of nitrogen (Stanier *et al*. 1971). The flasks were incubated at 28±2^{0}C under 3000 lux light intensity provided with cool fluorescent tubes and 16/8 hr light and dark cycle. Isolation and purification of cyanobacterial forms was carried out by repeated sub culturing followed by dilution and streak plate method. The identification was done following the keys given by Desikachary (1959).

The cyanobacterial isolates were examined for growth in terms of dry weight (Sorokin 1973) and pigments like chlorophyll (Mckinney 1941), carotenoids (Jensen 1978) and phycobiliproteins (Bennett and Bogorad 1973). In addition, total soluble proteins (Lowry *et al*. 1951), carbohydrates (Spiro 1966) and nitrogen

*Corresponding author, E-mail: dollywattaldhar@yahoo.com

fixing potential as acetylene reduction assay (Hardy *et al*. 1973) were also determined. All the experiments were replicated three times and homogenized samples were drawn during exponential phase of growth (15th day of incubation) for analysis.

Pure cyanobacterial isolates were identified and submitted in the germplasm of Centre for Conservation and Utilisation of Blue Green Algae (Table 1). Singh (1950) and Talpasayi (1962) made a systematic enumeration of cyanobacteria collected from moist soils and rocks. Distributional profile of cyanobacterial isolates from soils of Bhubaneshwar, Cuttack and Howrah indicated the predominance of heterocystous strains. Earlier workers have also shown the occurrence of mostly heterocystous forms due to their competitive ability in comparison to non-heterocystous forms (Garcia- Pichel and Belnap 1996). Singh (1971) also noticed that dominating nitrogen fixing blue green algae were heterocystous species of *Aulosira, Cylindrospermum, Nostoc, Anabaena, Tolypothrix* and *Calothrix* from soils of Cuttack, Orissa. Few non-heterocystous strains were also recorded from the soil samples collected from these areas.

A wide variation was recorded with respect to dry weight, acetylene reduction assay, pigments, total soluble proteins and carbohydrates amongst the isolates examined. The dry weight varied from 12.93 µg ml^{-1}(B-8) to 10.14 µg ml^{-1} (B-11) in the isolates from Bhubaneshwer. The cyanobacterial isolates from Cuttack showed a dry weight variation from the highest 14.52 µg ml^{-1} (C-11) to the lowest 9.43 µg ml^{-1} (C-6). Amongst the isolates from Howrah, *Nostoc* sp. strain H-2 showed highest dry weight (14.47 µg ml^{-1}) and strain H-1 exhibited lowest dry weight (9.8 µg ml^{-1}). The acetylene reduction assay (nmol C_2H_4 g^{-1}chl h^{-1}) varied from highest 0.92 in strain B-10 to the lowest 0.12 in strain B-3 amongst the isolates from Bhubaneshwer. On the other hand, the nitrogenase activity varied from 1.27 nmol C_2H_4 g^{-1}chl h^{-1} to 0.11nmol C_2H_4 g^{-1}chl h^{-1} in the isolates C-11 and C-1 respectively from the soils of Cuttack. Further, the nitrogenase activity was highest in Howrah strain H-6 (0.98 nmol C_2H_4g^{-1}chl h^{-1}) in comparison to other Howrah isolates examined (Fig. 1).

Table 1. List of cyanobacterial isolates from soils of Orissa and West Bengal

Strain	Code
Cuttack (Orissa)	
Calothrix marchica	C-1
Westiellopsis prolifica	C-2
Calothrix sp.	C-3
Lyngbya birgii	C-4
Westiellopsis prolifica	C-5
Anabaena variabilis	C-6
Calothrix javanica	C-7
Calothix sp.	C-8
Nostoc sp.	C-9
Westiellopsis prolifica	C-10
Lyngbya sp.	C-11
Calothrix sp.	C-12
Anabaena sp.	C-13
Nostoc sp.	C-14
Anabaena sp.	C-15
Bhubaneshwar (Orissa)	
Anabaena variabilis	B-1
Calothrix sp.	B-2
Hapalosiphon sp.	B-3
Hapalosiphon sp.	B-4
Microchaete sp.	B-5
Westiellopsis prolifica	B-6
Calothrix sp.	B-7
Nostoc sp.	B-8
Hapalosiphon sp.	B-9
Hapalosiphon sp.	B-10
Nostoc sp.	B-11
Calothrix sp.	B-12
Howrah (West Bengal)	
Anabaena sp.	H-1
Nostoc sp.	H-2
Calothrix sp.	H-3
Cylinderospermum sp.	H-4
Hapalosiphon sp.	H-5
Hapalosiphon sp.	H-6
Nostoc sp.	H-7

The nitrogenase activity is predominantly obtained in heterocystous forms as these are the sites for nitrogen fixation and vegetative cells can exhibit nitrogenase activity under microaerophilic conditions (Stewart 1971). Similarly, the variation in the trend of photosynthetic pigments (chlorophyll, carotenoids and phycobiliproteins) were noted amongst the cyanobacterial isolates tested.

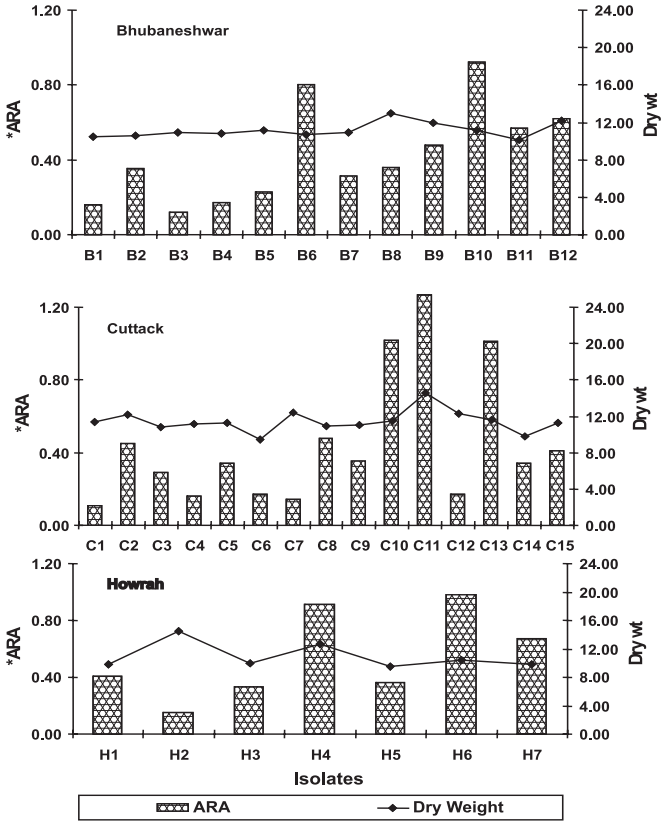

Fig. 1. Comparative nitrogenase activity (n mol C_2H_4 g^{-1} chl h^{-1}) and dry weight (mg ml^{-1}) amongst cyanobacterial isolates from Orissa and West Bengal, *ARA (acetylene reduction assay)

Chlorophyll and carotenoids remained low and exhibited more or less similar pattern in the isolates from these areas. In cosmetic industries, carotenes can be used in hand creams for sun rashes, nail polishes, lipsticks and anti wrinkle creams (Ben-Amotz *et al*. 1982). There have been a number of reviews published regarding the applicability of phycobiliproteins (Glazer & Stryer 1984, Jessby 1988). These photosynthetic pigments are used as natural protein dye in food industry (c-phycocyanin) because of their intense color, high solubility in water and their stability to change in pH (Arad 1988). These are also reported to have application in cosmetic industry (c-phycocyanin and c-phycoerythrin), as tracers in fluorescence immunoassays and in microscopy for diagnostic and biomedical research due to their high absorbance and reddish fluorescence (Tandeau de Marsac *et al*. 1993). Strain B-7, isolated from soils of Bhubaneshwer showed maximum phycobiliproteins (229.61 µg ml^{-1}) whereas, strain C-8 isolated from soils of Cuttack showed the highest level of phycobilliproteins (146.7 µg ml^{-1}). On the other hand, strain H-2 from Howrah showed maximum phycobilliproteins of 106.9 µg ml^{-1} (Fig. 2). Phycocyanin from *Spirulina* has been commercialized by "Dainippon Ink and Chemicals" of Japan under trade name "Lina Blue" which gives brilliant blue color with faint reddish fluorescence (Richmond 1990).

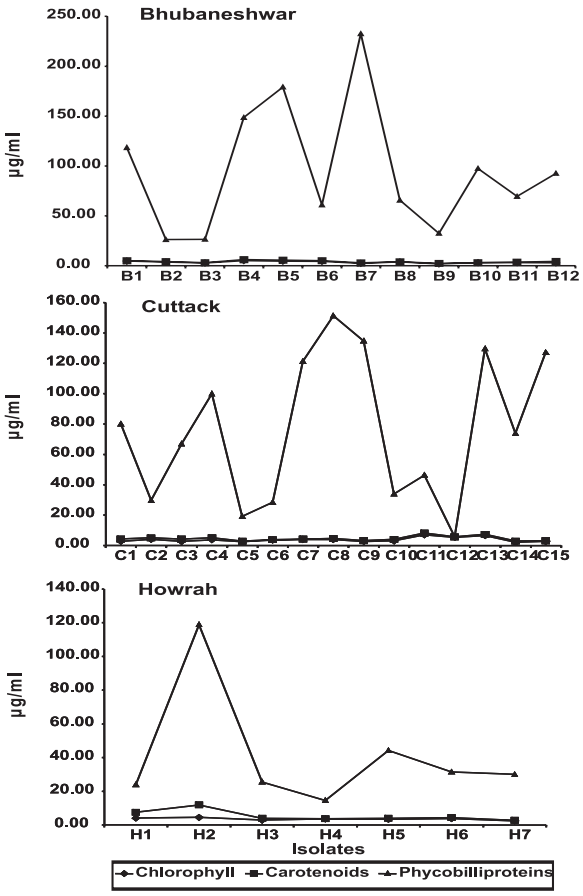

Fig. 2. Comparative photosynthetic pigments (µg ml^{-1}) amongst cyanobacterial isolates from Orissa and West Bengal

Strains B-9 and B-10 from Bhubaneshwer showed highest carbohydrates and total soluble proteins respectively, while strain C-11, an isolate from Cuttack showed maximum total soluble proteins (117.71 µg ml^{-1}) and carbohydrates (365 µg ml^{-1}). Interestingly, the strain H-2 from Howrah showed maximum total soluble proteins (122.57 µg ml^{-1}) as well as carbohydrates (331.33 µg ml^{-1}) (Fig. 3). Variability with respect to various parameters examined indicated that the organisms have simple metabolic requirements and are known to occupy wide range of niche (Reynolds 1987, Pearl 1988). The variability observed was in accordance with results obtained from earlier studies (Dhar *et al*. 2000, Mishra *et al*. 2001). Characterization of blue green algal strains has also been carried out for specific parameters in relation to their utilization in value addition (Garcia-Pichel and Castenholz 1991). Based upon the results obtained, these organisms can be examined for commercial viability as these are known to have immense biotechnological applications (Jensen 1978).

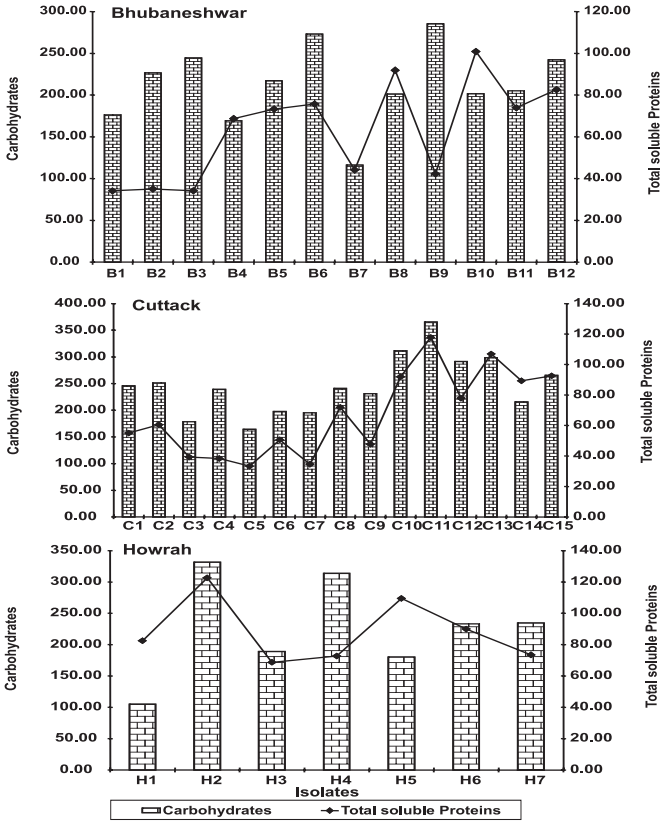

Fig.3. Metabolites (mg ml^{-1}) amongst cyanobacterial isolates from Orissa and West Bengal

REFERENCES

Arad, S (1988). Red microalgae as a source of biochemicals: Strategies for outdoor cultivation. In: S. Miyachi, I. Karube and Y. Ishida (eds.), Current topics in Marine Biotechnology, pp. 139-142. The Japanese Society for Marine Biotechnology, Tokyo.

Bennett, A. and Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue green algae. *J. Cell Biol.* **58:** 419-435.

Ben Amotz, A and Avron, M. (1982). Accumulation of β-carotene rich globules from *Dunaliella bardanil* (Chlorophyceae). *J. Phycol.* **18:** 529-537.

Desikachary, T.V. (1959). Cyanophyta. Indian Council of Agricultural Research, Publication, New Delhi.

Dhar, D.W., Tiwari, O.N., Prasanna, R., Pabbi, S. and Singh P.K. (2000). In: P.K. Singh, D.W.Dhar, S. Pabbi, R. Prasanna and A. Arora (eds.), Biofertilizers: Blue green Algae and Azolla , pp.24-54. Venus Printers and Publishers, New Delhi.

Garcia Pichel, F. and Castenholz, W.R. (1991). Characterization and biological implications of *Scytonemin*, a cyanobacterial sheath pigment. *J. Phycol*. **27:** 395-409.

Garcia-Pichel, F. and Belnap, J. (1996). Microenvironments and micro scale productivity of cyanobacterial desert crusts. *Phykos* **32:** 774-778.

Glazer, A.N and Stryer, L. (1984). Phycofluor probes. *Trends Biochem. Sci*. **9:** 423-427.

Gupta, D. (1975). Some new records of blue green algae from West Bengal. *Bull. Bot. Soc. Bengal* **19:** 1-2.

Hardy, R.W.F., Burns, R.C and Holsten, R.D. (1973). Application of acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem*. **5:** 47-81.

Jessby, A. (1988). *Spirulina* model for micro algae as human food. In : C.A. Lambi , J. R. Wealand (eds.), Algae and Human Affairs, pp. 149-179. Cambridge University Press. Cambridge.

Jensen, A. (1978). Chlorophylls and carotenoids. In: J.A. Hellebust, J.S. Craige (eds.), Phycological Methods: Physiological and Biochemical methods, pp.59-70. Cambridge University Press, Cambridge.

Kolte, S.O. and Goyal, S.K. (1985). Distribution pattern of blue green algae in rice field soils of Vidarbh region of Maharashtra state. *Phykos* **24:** 156-162.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin phenol regent. *J. Biol. Chem.* **193:** 262-275.

McKinney, G. (1941). Absorption of light by chlorophyll. *J. Biol. Chem.* **140:** 315-322.

Mishra, U., Pabbi, S. and Singh, P.K. (2001). Cyanobacterial diversity in tarai belt of Uttar Pradesh, India: Occurrence of some heterocystous forms. *Phykos*. **40:** 89-94.

Paul, T.K. and Santra, S.C. (1982). Contributions to the cyanophyceae of Murshidabad. *Phykos* **21:** 150-152.

Pearl, H.W. (1988). Growth and reproductive strategies of fresh water blue green algae. In: C. Sandgreen (ed.), Growth and Reproductive Strategies of Fresh Water Phytoplankton, pp. 261-315. Cambridge University, U.K.

Reynolds, C.S. (1987). Cyanobacterial water blooms. In: J.A. Callow (ed.), Advances in Botanical Research, Vol. 13, pp. 67-143. Academic Press, London.

Richmond, A. (1990). *Spirulina*. In: M.A. Borowitzka and L.J. Borowitzka (eds.), Microalgae Biotechnology, pp. 85-121. Cambridge University Press, Cambridge.

Singh, P.K. (1985). Nitrogen fixation by blue green algae in paddy fields. In: Rice Research in India. Indian Council of Agricultural Research Publication, New Delhi.

Singh, R.N. (1950). Reclamation of usar lands in India through blue green algae. *Nature* **165:** 325-326.

Sinha, J.P. and Mukherjee, D. (1975). Blue green algae from the paddy fields of Bankura district of West Bengal, *Phykos* **14:** 117-118.

Sorokin, C. (1973). Dry weight: packed cell volume and optical density. In: J.R. Stein (ed.), Handbook of Phycological Methods, Culture Methods and Growth Measurements pp. 321-343. Cambridge University Press, London.

Spiro, R.A. (1966). Analysis of sugars found in glycoproteins. In: E.F. Neufeld and V. Ginsburg (eds.), Methods in Enzymology VIII, pp. 3-26. Academic Press, New York.

Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen Bazire, G. (1971). Purification and properties of unicellular blue green algae, order chroococcales. *Bacteriol. Rev.* **3:** 171-205.

Stewart, W. (1971). Physiological studies on nitrogen fixing blue green algae. *Plant Soil* (special volume): 377-391.

Talpassayi, E.R.S. (1962). The myophyceae of Kumaon hills, Uttar Pradesh, India. *II Proc. Indian Acad. Sci*. **B55:** 251-255.

Tandeau de Marsac and Houward, J. (1993). Adaptation of cyanobacteria to environmental stimuli: new steps to world's molecular mechanism. FEMS *Microbiol. Rev.* **104:** 119-190.

Venkataraman, G.S. (1975). The role of blue green algae in tropical rice cultivation. In: W.D.P. Stewart (ed.), Nitrogen Fixation by Free-Living Microorganisms, pp. 207-218. Cambridge Univ. Press, London.