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COMPARATIVE STUDY OF SOME NITROGEN-FIXING BLUE-GREEN ALGAE OF MULTAN (PAKISTAN)

J.I.MIRZA, M. KHALID AND M.H. JAVED*

Institute of Pure and Applied Biology (Botany Division), and Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.

Abstract

Naturally growing blue-green algal samples collected from Multan were cultured in nitrogen free medium and of the 7 species isolated in unialgal culture, 6 species belonged to heterocystous genera viz., *Nostoc, Anabaena* and *Calothrix* with a non-heterocystous genus *Lyngbya*. During 52 days incubation, the total nitrogen fixed by *Anabaena iyengarii* was 11.45 mg being the maximum among all the species tested with a minimum amount of 0.31mg fixed by *Lyngbya spiralis*.

Introduction

Pakistani soil being extremely poor in nitrogen is a major factor limiting the production of agricultural crops (Wahab, 1967). Soaring costs of commercially produced nitrogen fertilizers and potential pollution hazards due to indiscriminate use of fertilizers (Okon & Hardy, 1983) have stimulated a search for alternative source of nitrogen where the biological role played by the nitrogen fixing prokaryotes i.e., blue-green algae and bacteria has great importance. Compared to the non-photosynthetic bacteria, the blue-green algae can fix both carbon dioxide and nitrogen. De (1939) first recognized the importance of nitrogen fixing blue-green algae in paddy fields of India as the factor responsible for maintaining soil fertility in terms of nitrogen. Work on the nitrogen fixation by the blue-green algae have been made (Paerl *et al.*, 1981; Watanabe & Brotonegoro, 1981; Okon & Hardy, 1983). Although Blue-green algae have been reported from various parts of Pakistan (Ali *et al.*, 1978; Hussain *et al.*, 1984; Anjum & Faridi, 1985), however, there has been only one report (Ali & Sandhu, 1976) describing the laboratory cultivation of blue-green algae on nitrogen free medium. The present paper describes nitrogen fixation by 7 species of blue-green algae from Multan.

Materials and Methods

Blue-green algal samples were collected from various localities of Multan in November, 1986. Each sample was then used as inoculum of 3mm diameter into 25ml of nitrogen free growth medium in 9cm diameter glass Petri dishes. The nitrogen free growth medium was prepared according to the method of Meidner (1984), containing 40mg K_2HPO_4, 75mg $MgSO_4$, $7H_2O$, 36mg $CaCl_2.2H_2O$, 6mg ferric citrate, 2.86mg H_3BO_3,

*Present address and Address for correspondence: Department of Biochemistry, The Aga Khan University Medical College, P.O. Box 3500, Stadium Road, Karachi, Pakistan.

1.81mg $MnCl_2.7H_2O$, 0.390mg $NaMoO_4.2H_2O$, 1mg EDTA (tri Na-salt), 20mg Na_2CO_3, 0.079mg $CuSO_4.5H_2O$, in 1 liter distilled water. After sterilization, the pH was adjusted to 7.3 with KOH.

For unialgal culture, the isolation of algae used as inoculum was done using the method of Ali & Sandhu (1976). The algal material thus isolated was transferred into sterile nitrogen-free growth medium in a Petri dish with 2 replicates and incubated between 25-30°C with 12h daily illumination. After 52 days the cultures were centrifugated at 3000 rpm for 10 min and fresh weight of algal mass calculated.

For each species, the amount of nitrogen in the algal mass and the supernatant was estimated separately by Nesslerization method (Varley *et al.*, 1980) using 10mg algal mass in a test tube mixed with 0.1ml acid digestion mixture (28% H_3PO_4, 0.28% $CuSO_4.5H_2O$ and 10.8% H_2SO_4) and 1 crystal of selenium dioxide. The contents were then digested directly by heating on a flame. After digestion, 0.1ml of 1% gum arabic solution was added at room temperature. The volume was then made upto 3.5ml with distilled water and 1.5ml Nessler's reagent was added. Three ml of supernatant was treated in similar fashion for Nesslerization. The Nesslerized solutions were read at 500nm on a Shimadzu UV Visible Recording Spectrophotometer with $(NH_4)_2SO_4$ used as a standard. The amount of nitrogen in the sample was calculated:

$$\text{Nitrogen (mg)} = \frac{\text{O.D. of the unknown sample}}{\text{O.D. of the standard sample}} \times \text{Concentration of the Standard}$$

Total nitrogen fixed is expressed as mg/25ml of culture medium.

Results and Discussion

On nitrogen free medium, the highest growth (3.42gm) was of *Lyngbya spiralis*. Since a part of the fixed nitrogen is excreted into the surrounding medium (Tyagi & Kumar, 1980), the nitrogen fixing capacity of each species was estimated from both the algal mass and the supernatant solution. The highest amount of nitrogen (11.45mg) was fixed by *A. iyengarii* and the lowest (0.31mg) by *L. spiralis* (Table1). Nitrogen fixation by heterocystous filamentous forms is well known, but there are only few reports of nitrogen fixation by non-heterocystous filamentous (Watanabe & Brotonegoro, 1981: Okon & Hardy, 1983). The heterocysts, being non-photosynthetic, possess reductive intracellular environment and are, therefore, the main sites of nitrogen fixation. The heterocystous forms can, therefore, fix nitrogen under aerobic conditions (Paerl *et al.*, 1981). On the other hand, non-heterocystous forms have been shown to fix nitrogen only under anaerobic or semi-aerobic conditions (Watanabe & Brotonegoro, 1981) which could be the reason for low nitrogen fixation by *L. spiralis* a well-known non-hetero-

Table 1. Growth and nitrogen fixation by different blue-green algae after 52 days incubation.

Algae	Algal Fresh weight* (gm)	Total Nitrogen fixed** (mg)
Lyngbya spiralis Geitler	0.31	0.31
Nostoc punctiforme (Kuetz.) Hariot	2.08	5.30
Anabaena variabilis Kuetz. ex Born. et Flah	2.75	9.37
Anabaena iyengarii Bharadwaja	3.42	11.45
Anabaena spiroides Klebahn	2.55	8.18
Galothrix elenkinee Kossinskaja	1.10	6.43
Calothrix marchica Lemmermann	0.77	3.16

Each algal species was inoculated into 25ml nitrogen free medium in 9cm Petri dishes.
*The weight determined after centrifugation and separation of the supernatant.
** The nitrogen determined from the algal mass and the supernatant.

cystous form in our culture conditions. Blue-green algae are salt tolerant and have been claimed to be successfully used for the reclamation of alkaline 'Usar' soil in India (Singh, 1961). A search for the blue-green algae showing higher rates of nitrogen fixation would be useful for the improvement of nutritionally poor saline soils in Pakistan.

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References

Ali, S. and G.R. Sandhu. 1976. Laboratory cultivation of blue-green algae of saline soils and rice fields. *Proc. Nat. Panel on Algae and its utilization*. pp. 553-57.

Ali, S., M.I. Rajoka and G.R. Sandhu. 1978. Blue-green algae of different rice growing soil series of the Punjab. *Pak. J. Bot.*, 10: 197-207.

Anjum, G. and M.A.F. Faridi. 1985. Algae in dry soils of N.W.F.P., Pakistan, *Pak. J. Bot.*, 17: 257-261.

De, P.K. 1939. The role of the blue-green algae in nitrogen fixation in rice fields. *Proc. Roy. Soc. London.* Ser. B, 127: 121-139.

Hussain, F., G. Anjum, M.I. Zaidi and M.A.F. Faridi. 1984. Fresh water algae of Hanna Urak Valley, Quetta, *Pak. J. Bot.*, 16: 81-84.

Meidner, H. 1984. *Class Experiments in Plant Physiology*. George Allen and Unwin Pubs, UK.

Okon, Y. and R.W.F. Hardy. 1983. Development in basic and applied biological nitrogen fixation. In: *Plant Physiology-A Treatise (*Ed.), F.C. Steward, pp. 5-54, Academic Press.

Paerl, H.W., K.L. Webb, J. Baker and W.J. Weibe. 1981. Nitrogen fixation in waters. In: *Nitrogen Fixation, Vol. 1: Ecology*. (Ed.) W.J. Broughton, pp. 193-241, Charendon Press, Oxford.

Singh, R.N. 1961. *Role of Blue-green Algae in Nitrogen Economy of Indian Agriculture*. Times of India Press, Bombay.

Tyagi, V.V.S. and H.D. Kumar. 1980. Dinitrogen fixation by blue-gren algae. In: *Ann. Rev. of Plant Sciences* (Ed.) C.P. Malik, pp. 1-36, Sheetal Press, New Delhi.

Varley, H., Gowenlock, A.H. and M. Bell. 1980. The plasma proteins. In: *Practical Clinical Biochemistry*, pp. 535-595, William Heinemann Medical Book Ltd., London.

Wahab, A., 1967. *Zamin aur Zaraat*, Chitan Press, Lahore.

Watanabe, I. and S. Brotonegoro. 1981. Paddy fields. In: *Nitrogen fixation, Vol. 1: Ecology* (Ed.) W.J. Broughton, pp. 242-263, Clarendon Press, Oxford.

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