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ACETYLENE REDUCTION ACTIVITY OF SOME BLUE-GREEN ALGAE

SURENDRA SINGH
Department of Biochemistry, North-Eastern Hill University, Shillong 793 014, India.

MOST aerobic nitrogen-fixing blue-green algae are filamentous forms and N_2 fixation takes place in differentiated cells called heterocysts which serve as N_2-fixing factories[1]. Certain N_2-fixing, heterocystous blue-green algae develop associations with algae, fungi, bryophytes, the water fern *Azolla*, gymnosperms and the angiosperm *Gunnera*[2]. These associations are of interest because the algae supply fixed nitrogen to the host plant and are, in many ways, analogous to rhizobia[3]. Relative to free-living isolates, symbiotically associated blue-green algae have a five-fold or even higher frequency of heterocysts[4,5]. The present paper describes the acetylene reduction activity (ARA) of six blue-green algae in relation to their heterocyst frequency and age. These estimates would serve as a reference for inoculum selection, growth and biochemical studies.

The blue-green algal isolates used are listed in table 1. Axenic cultures of the blue-green algae were grown in BG-11 medium[6] under continuous light at 50 μmol photons $m^{-2} \cdot s^{-1}$ at 25°C. The cultures were harvested after 3, 7 and 30 days, washed with sterile BG-11 medium[7] and suspended in the same medium. ARA was measured by the acetylene reduction assay[7]. The cultures (5 ml each) were placed in 15 ml serum vials with 10% (v/v) acetylene as the gas phase at 25°C and a photon flux of 250 μmol $m^{-2} \cdot s^{-1}$. Gas samples (1 ml) in two replicates were collected after 30 min and analysed in a gas chromatograph (model Tracor 540). The heterocyst frequency was calculated as per cent of total cells by light microscope observations of the filaments. Chlorophyll *a* was estimated by the method of Mackinney[8].

Table 1 shows the data on relative ARA and heterocyst frequency of six blue-green algal strains. Based on their ARA values and heterocyst frequency, the algae can be separated into two main groups: one with high ARA and high heterocyst frequency (*Nostoc anthoceros, Anabaena cycadeae*) and the other with low ARA and low heterocyst frequency (*Anabaena* 7120, *A. variabilis, A. doliolum* and *Nostoc linckia*). The free-living isolates from *Anthoceros* and *Cycas* had high ARA values (4.47 and 4.04 nmol C_2H_4 per μg Chl *a* per hour respectively) compared with the other isolates. This can be explained by the fact that these isolates had average

Table 1 *Acetylene reduction activity and heterocyst frequency of blue-green algae*

Isolate	Source	Acetylene reduction activity* (nmol C_2H_4 per μg Chl *a* per hour) Young culture (3-day)	log-phase culture (7-day)	old culture (30-day)	Heterocyst frequency (%)
Anabaena doliolum	Isolated from paddy field (BHU)	1.47	3.38	0 586	4.95
Anabaena 7120	Gift from B. Bergman (Uppsala, Sweden)	1.42	2.98	0.284	4.55
Anabaena variabilis	Gift from A. K. Kashyap (BHU)	1.29	3.39	0.691	4.65
Nostoc linckia	—"—	1.51	3.60	0.713	4.72
Anabaena cycadeae	Original isolate from corralloid root of *Cycas circinalis* (BHU)	1.71	4.04	1.29	6.00
Nostoc sp.	Original isolate from *Anthoceros* gametophyte (Shillong)	1.88	4.47	1.59	8 90

*Average of six independent experiments.

heterocyst frequency of 8.9 and 6% respectively, compared with values of 4–5% in the other isolates. This observation further substantiates the importance of heterocysts in nitrogen fixation. Fairly young (3-day) and very old (30-day) cultures showed lower ARA than those in log phase (7-day), suggesting that the enzyme nitrogenase is synthesized significantly more in the log phase. ARA was also altered by changes in medium and culture conditions (data not shown), suggesting that the history of cultivation and age of cultures play an important role in the regulation of ARA (nitrogen fixation) in blue-green algae.

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TROPANE ALKALOIDS FROM *LYCIUM BARBARUM* LINN., *IN VIVO* AND *IN VITRO*

M. L. HARSH
Department of Botany, Dungar College, Bikaner 334 001, India.

TROPANE alkaloids, which are pharmacologically important compounds, have been reported from solanaceous plants such as *Atropa*[1,2], *Hyoscyamus*[3,4], *Datura*[5,6], *Duboisia*[7] and *Scopolia*[8]. However, there is no report on the production of tropane alkaloids from intact plant parts and in tissue culture of *Lycium barbarum* of an Indian arid zone. Therefore the present investigation was undertaken.

Plant materials (roots, shoots and fruits) were freshly collected from Davi Kund Sagar, Bikaner. The voucher specimen of *L. barbarum* was collected from the Department of Botany, University of Jodhpur, Jodhpur and deposited at the same place. Unorganized tissue of *L. barbarum* was established from seedlings on Murashige and Skoog's[9] medium supplemented with 5 ppm of kinetin. The growth index (GI) of each of the tissue samples was calculated from

$$GI = \frac{\text{Final dry weight of tissue} - \text{initial dry weight of tissue}}{\text{Initial dry weight of tissue}}$$

The various plant parts and 6-week-old tissues at maximum GI (7.5) were dried and ground to a fine powder. The powdered material was extracted with chloroform for 24 h. Thin-layer chromatography (silica gel G; chloroform: methanol: ammonia, 30:60:2) gave two spots in all the plant parts and tissue samples tested. The spots were visualised under UV light (254 nm) and developed by spraying with modified Dragendorff's reagent[10] and heating at 100°C until the characteristic colours developed. The spots corresponded with reference atropine (R_f 0.35, dark orange) and hyoscyamine (R_f 0.36, light orange). The alkaloids present in the extracts were obtained by preparative (0.4 to 0.5 mm) TLC on silica gel plates eluted with chloroform. Each isolated compound was crystallized and further analysed for m.p., m.m.p. and IR spectra, and compared with the authentic sample for confirmation. The various extracts were subjected to quantitative estimation by the procedure of Feldman and Robb[11] for atropine and the method of Gaur[4] for hyoscyamine. Five replicates were examined in each case, and mean and 95% confidence limits obtained.

The callus tissue was hairy, compact in texture, and whitish-green in pigmentation. The maximum GI observed was 7.5 in a six-week-old tissue. Tropane alkaloids in each of the plant parts and in 6-week-old tissue at maximum GI were identified as atropine and hyoscyamine. Atropine and hyoscyamine were confirmed by m.p. (atropine, 117–118°C and hyoscyamine, 108°C), m.m.p. (undepressed) and IR spectroscopy.

Total alkaloid was nearly the same in shoots and fruits lower in calli, and still lower in roots (table 1). Fruits had the highest atropine content and shoots the highest hyoscyamine content.