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Effect of Arsenate on Growth and Photosynthesis in Synechococcus PCC 7942

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Abstract*: The aim of the present study was to evaluate the effect of sodium arsenate on Synechococcus PCC 7942 in terms of total growth and photosynthesis. The number of cells in the culture was counted. Photosynthetic O_2 evolution, fluorescence excitation and emission spectra were recorded. Synechococcus cultures in the presence of various concentrations (1, 5, 10, 20 and 50 µM) of sodium arsenate exhibited decrease in the rate of growth and photosynthetic O_2 evolution and it is concentration dependent. Room temperature absorption and excitation spectra of cells revealed gradual loss of phycobilisomes (PBS) and Chlorophyll-a (Chl-a) component with increasing concentration of arsenate. In conclusion, Synechococcus PCC 7942 genus has its own defense mechanism for Arsenate resistance in natural habitat. Algal cells growing under extreme environment and also in polluted water have the capacity to adapt that environment.

Keywords: Arsenate, Cyanobacteria, Heavy metals, Photosynthesis, Synechococcus

I. INTRODUCTION

Blue green algae are prokaryotes that perform oxygenic photosynthesis [1]. Cyanobacteria are the scientific name for blue green algae or pond scum. Cyanobacterial cells resemble the chloroplasts of higher plants in terms of membrane structure, composition of membrane lipids and structure of the photosynthetic machinery [2, 3]. In present age of Industrialization and agriculture, like other organisms, Cyanobacterial cells are constantly facing different kind of stresses in their external and internal environment and are struggling to survive through morphological and metabolic alterations [4, 5]. Cyanobacterial research is drawing increased attention in biotechnology due to its beneficial role in a wide range of fields including heavy metal removal and recycling [6, 7]. Survival of organisms in metalliferous environment may be by compartmentalization of metals in cell wall, vacuoles and polyphosphate bodies [8]. Cyanobacteria act as a valuable model for the study of the molecular mechanisms involved in tolerance to environmental stresses including heavy metal stress [9]. Arsenic, a heavy metal is being released into the environment through industrial and agricultural activities [10-12]. Arsenic naturally occurs throughout the earth's crust, water, air, plants and animals. It is released into the environment due to volcanism, erosion of rocks, forest fires and also human industrial activity [13, 14]. Studies in China and elsewhere have shown that arsenic trioxide is effective in treating acute promyelocytic leukaemia [15, 16]. The phytotoxic impact of arsenic was well documented in certain plant systems [17-19]. However the paucity of information on the physiological effects of arsenic in plant systems prompted to take up the present endeavour. The objective of the present study is to assess the impact of arsenic on *Synechococcus* as a test system.

II. MATERIALS AND METHODS

A. Algal cultures

The prokaryotic unicellular blue green algae *Synechococcus* PCC 7942 were grown in BG11 medium. The organism was grown at still cultures under 20 $W.m^{-2}$ at 25 °C in conical flasks (250, 500 and 1000 ml).

B. Growth measurement - Direct cell count

The number of cells in the culture at different days was counted by using Neubauer Haemocytometer.

C. Measurement of O_2 evolution

Photosynthetic O_2 evolution was monitored at intervals of 2 days at 25 °C in a clark type O_2 electrode hooked to a recorder. The reaction was carried out at 10 µl volume. Saturating white light at 200 $W.m^{-2}$ was provided by a slide projector and was passed through a 15 cm long filter of 1% $CuSO_4$ solution.

D. Fluorescence and excitation spectrum

Fluorescence excitation and emission spectra were recorded using a Hitachi MPF4 spectrofluorimeter. When the excitation spectra were recorded, the slit widths of the excitation and emission monochromators were kept at 5 and 10 nm, respectively. Cells were suspended at Chl-a concentration of 2 µg/ml and placed in 1.0 ml cuvette to avoid self absorption.

E. Absorption spectrum

Room temperature absorption spectra were followed in a Hitachi 557 spectrophotometer. Cells were placed in 2 sides' ground quartz cuvette and the ground side was placed in the optical path to diffuse the reference and sample beams equally.

III.RESULTS AND DISCUSSION

A. Synechococcus culture growth

With the main objective as to study the effect of arsenate on the growth and photosynthetic activities, common Cyanobacterium *Synechococcus* cultures were grown autotrophically in the presence and absence of various concentrations of sodium arsenate. Initial experiments were carried out to find out the range of concentration that produces different levels of inhibition. Concentration of 1, 5, 10, 20 and 50 µM was therefore selected.

Culture growth under control conditions showed an initial log period of 2 days and therefore rapid increase in growth (Fig. 1). A similar trend was observed in the presence of 1, 5 and 10 µM arsenate. However higher arsenate concentration 20 and 50 µM caused an inhibition in the cell growth during the first 2 days followed by a slow recovery. Such recovery was very slow in the presence of 50 µM arsenate. In this case although high arsenate concentration had caused inhibition in cell growth, slowly adapted to high arsenate concentration. This could be either due to partial metabolism of arsenate in the medium or through modification of cellular components [20].

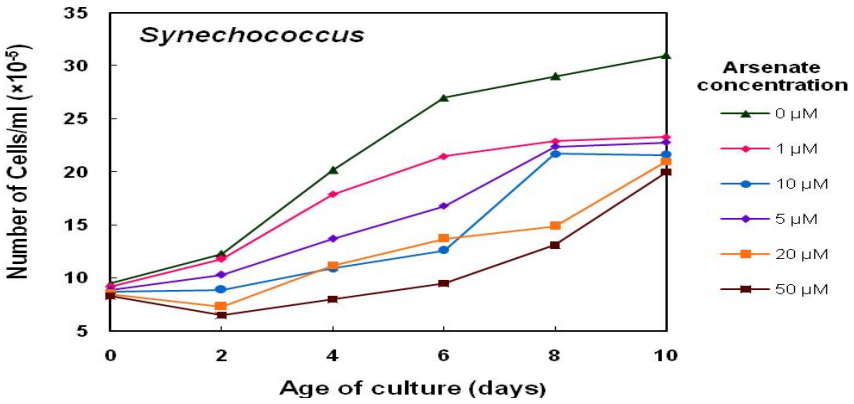

Fig. 1 Changes in the growth of *Synechococcus* cultures in the presence of various concentration of sodium arsenate

B. Rate of photosynthetic oxygen evolution in Synechococcus cells

To find out the effect of arsenate on primary metabolic activity of the cells namely the photosynthesis, changes in the rate of O_2 evolution as a function of arsenate concentration were followed. With increase in the concentration of arsenate, the rate of O_2 evolution was inhibited more or less linearly (Fig. 2). Even with the maximum concentration tried (50 µM) the O_2 evolution capacity had decreased only by 50 %.

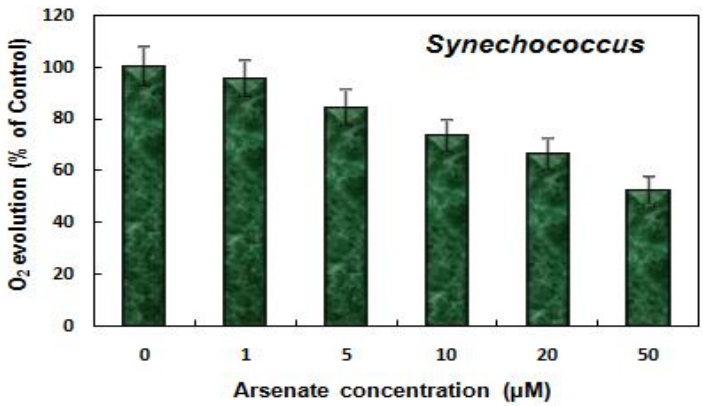

Fig. 2 Changes in the rate of photosynthetic oxygen evolution in *Synechococcus* cells collected from 5 day old cultures grown in the presence of various concentration of sodium arsenate

C. Absorption and difference spectra of Synechococcus cells

Since the loss of photosynthetic activity could be due to various factors, detailed investigations on the changes in the light absorbing chromophores and chlorophyll were followed [21]. Changes in the room temperature absorption and difference spectra of *Synechococcus* cells collected from 5 day old cultures grown in the presence of various concentration of sodium arsenate are shown in fig. 3-4. The control cells showed absorption maxima at 620 nm and 678 nm (Fig. 3). With increasing concentration of arsenate, there is a gradual decrease in the level of these absorption bands. At the same time, a prominent shoulder appeared at 645 nm. These changes are clearly revealed in the absorption spectra (treated minus control cells) spectra. When normalized at 655 nm, prominence of the bands at 585, 620, 642 and 672 nm are evidenced in arsenate treated cells.

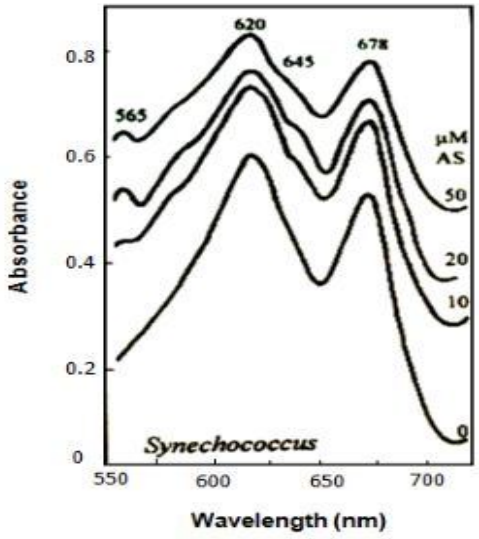

Fig. 3 Changes in the room temperature absorption spectra of *Synechococcus* cells

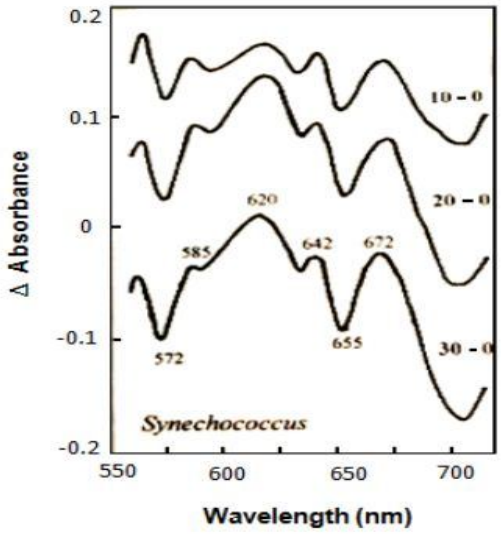

Fig. 4 Changes in the room temperature absorption difference spectra of *Synechococcus* cells

D. Fluorescence excitation spectra of Synechococcus cells

In the absence of any drastic loss in the primary and secondary light absorbing components, it is of interest to know whether the light mediated initial reaction of photosynthesis are affected [22]. For this excitation spectra of these cells were followed. Fluorescence excitation spectrum of untreated cells showed strong excitation at wavelengths with peak at 620 nm indicating the large contribution of PBS. Changes in the room temperature fluorescence excitation spectra for F-682 in *Synechococcus* cells

collected from 5 day old cultures grown in the presence of various concentration of sodium arsenate are shown in (Fig. 5). Arsenate at different concentration did not produce any large decrease in the excitation band of chl-a or phycobilins.

The relative contribution of chlorophyll and PBS to the photosynthesis is expressed in terms of 620/440 nm ratio (Table 1). Although the fluorescence levels at 440 and 620 nm varied in arsenate treated cells strong excitation was maintained even with the highest concentration of arsenate in these experiments. This indicates that the energy transfer from the PBS to Chl [23, 24].

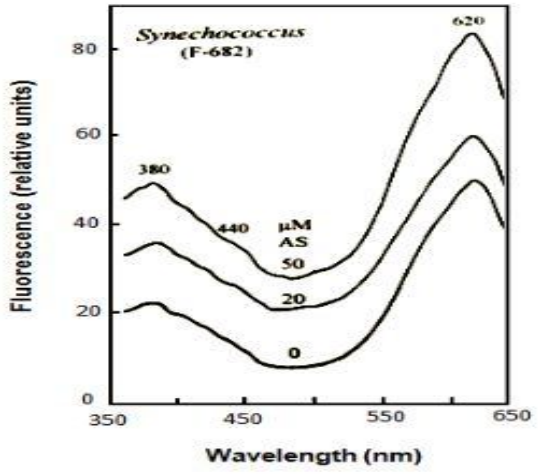

Fig. 5 Changes in the room temperature fluorescence excitation spectra for F-682 in *Synechococcus* cells

TABLE 1
Changes in the 620/440 fluorescence ration in Synechococcus cells collected from cultures grown in the presence of various concentration of sodium arsenate

Arsenate Concentration (µM)	Relative fluorescence level		620/440 nm Ratio
	440 nm	620 nm	
0	10	64	6.4
10	12	71	5.9
20	11	70	6.3
50	9	64	7.1

Values were obtained from similar traces shown in Fig. 5.

IV. CONCLUSIONS

In conclusion, Sodium arsenate at various concentrations (1, 5, 10, 20 and 50 µM) showed decrease in *Synechococcus* culture growth and O_2 evolution and the extent of reduction is concentration dependent. The early growth of cultures was affected by arsenate at 20 and 50 µM concentration and the cultures recovered slowly thereafter. Room temperature absorption spectra and fluorescence excitation spectra revealed strong contribution of phycobilisomes to photosynthetic process.

V. ACKNOWLEDGMENT

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