ISSN: 2456-7744

Advanced Scientific Research 2017 2(1) 6-7

Advanced Scientific Research
journal homepage: www.advancedscientificresearch.in

Effect of herbicides on some selected cyanobacterial species

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ARTICLE INFO

Article history:
Received 24 May 2017
Received in revised form 25 June 2017
Accepted 28 June 2017
Available online 29 June 2017

Keywords:
Bioenergetic studies
Oxygen evolution studies
Cyanobacteria
Spheroplast

ABSTRACT

Attempts had been made to improve the sensitivity of whole cells of cyanobacterial species and its thylakoidal fragments where PSII is the main target for most of the photodynamic herbicides. We screened out the potential of tolerance. These biologically active materials are capable of oxygen evolution by photolysis of water in presence of light. We performed growth studies, pigment analysis, bioenergetic studies by using clark's oxygen electrode both in free and immobilized state. We recorded spectral properties, oxygen evolution studies in presence of various herbicides. We determined the I50 values for the whole cells of different species and also recorded peak shifting.

1. Introduction

Heavy metal ion pollution resulting from industrialization and excess use of fertilizers, herbicides and pesticides are reasons for heavy metal toxicity in both aquatic and terrestrial environment [1,3-4]. The toxic level of these pollutants in water bodies affects mostly the photosynthetic processes [5-7]. Current assumptions are that the changes in global climatic pattern due to green house effect would also alter the distribution of these pollutants in the soil surface. Herbicides, heavy metals and pesticides can be highly toxic to human and animal health; their jumbled use has serious environmental insinuation. To monitor such low residual levels of the herbicides there is a need for developing sensitive and reliable detection methods. About one half of the herbicides presently used in agriculture inhibit the light reactions in photosynthesis, mostly by targeting the photosystem II (PSII) complex[8-12]. In PSII, the D1 protein is the main target of the herbicides. It is clear that herbicides are able to inhibit the Hill reaction in isolated chloroplasts and spheroplast[13,14]. On the basis of this strategy, thaylakoidal fragments/spheroplasts have been used, conversely, to detect herbicides by testing inhibition of the Hill reaction and inhibition of DCPIP photoreduction[20-22]. Currently, some methods which are generally used in the testing of most herbicides are HPLC, GC-MS and ELISA[17,18].

2. Experimental

Preparation of culture and optimization of growth conditions of the cultures: Various strains of cyanobacteria i.e., *Spirulina platensis*, *Synechococcus PCC 7942* and *Nostoc muscorum* were collected from different laboratories including National Phytron Facility Centre, IARI, New Delhi. Stock cultures were maintained in a culture room continuously illuminated with cool fluorescent light (70 µM sec-2) at 25±2°C in 16 h light and 8 h dark conditions. All the culture flasks were continuously bubbled with air using air pump. The cultures were maintained under bacteria free conditions by regularly transferring the exponentially growing cultures to fresh sterile growth medium. We provided them a varying range of pH, temperature and light and recorded the best conditions for optimal growth.

Screening of different cyanobacterial species for testing their response towards different types of herbicides: We used various concentrations of herbicides, 2, 4 D, Diuron and DCMU for testing the inhibitory potential of the 3-cyanobacterial species. Varying concentrations of 2,4 D, 25, 50, 100, 200, 500, 600 and 700 µM; DCMU, 20, 40 and 60 µM and Diuron, 200 and 400 µM were taken. Readings were taken at 0th, 12th, 24th, 36th and 48th h. All the experiments were performed in triplicate sets.

Spheroplast preparation: Cells were first harvested by centrifuging them at 9000 g for 5 min. Pellet was washed once with spheroplast preparing buffer (Tricine-KOH (pH-7.5) containing 500 mM sucrose, 10 mM KCl and 10 mM EDTA) and resuspended in the same buffer. This suspension was incubated at 37°C in a water bath for 3 h with lysozyme (1 mg mL-1). After 3 h the reaction was stopped by centrifuging the suspension. The supernatant was discarded and the pellet was resuspended in the same buffer and processed for further work [15- 23].

Thylakoid isolation: Cells were harvested by centrifugation at 5000 g for 20 min and the pellet was suspended in 75 mM Tricine buffer containing 10 mM NaCl (pH-7.5). Cells were disrupted by ultrasonication for 4x15 sec periods and punctured by 15 sec rest periods in an ice bath. Unbroken cells were removed by spinning at 2500 g for 30 min to get a pellet of cell wall fragments. The resulting supernatant was spun at 35,000 g for 30 min at 4°C to sediment the thylakoids[16-24].

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Immobilization: The photosynthetic material was immobilized in an albumin glutaraldehyde matrix (BSA-GA)[2-11]. All steps were performed at 4°C under a dim green light. The procedure involved mixing of 60 mM sodium phosphate buffer at pH 7.4, 15% BSA solution and 1.5% glutaraldehyde solution. The mixture was incubated for 2 min and then the photosynthetic material was added which was followed by 5 sec of agitation. This mixture was immediately distributed into working electrode. The electrode with immobilized samples were kept at -20°C for a minimum time of 12 h.

3. Results and Discussion

The growth optimization conditions for *Nostoc muscorum* and *Synechococcus PCC 7942*, was pH 7.8, light intensity of 70-80 µM sec-2 and CO_2 rich air supplement of 5% (V/V) with rotation of 160 rpm. We also observed that at certain concentration of 2, 4 D (10-4 M), growth was promoted instead of showing inhibitory effect, earlier work done supported our results [11]. It was found that in case of *Spirulina platensis* the inhibitory effect was observed only from 8 mM concentration. The concentration of 600 µM and above was inhibitory to *Nostoc muscorum* and *Synechococcus PCC 7942*. This study showed that the rigid cell wall of *Synechococcus PCC 7942* did not show response during short incubations with the herbicides, our results showed the similarity with earlier work done[16]. Hence, we used spheroplasts and thylakoidal fragments to improve the sensitivity of the biomaterial towards the stress. UV-Vis spectrophotometric analysis provided us the basic spectral properties of cyanobacteria[15]. We observed four types of major peaks, one peak of chlorophyll a in red light region (680 nm) and trace of chlorophyll a in blue light region (440 nm), one peak of carotenoid (490 nm) and one peak of phycobilisomes (650 nm in our case), our results showed close similarity with earlier work done[5]. All the peaks were greatly altered and showed peak shifting and changes in optical densities during the experiments with respect to the control. When the samples were treated with 2, 4 D, Diuron and DCMU, there was clear peak shifting and changes in the intensities. We performed two types of studies; one involving long incubation of the whole cells with the herbicides and secondly, short incubation using spheroplasts and thylakoidal fragments. We immobilized the samples directly on working electrode and recorded the amount of oxygen evolution with respect to control. There was a slight decrease in activity using immobilized sample, work done earlier supported our results[21-25]. This technique will be exploited to design a biosensor for commercial use.

4. Conclusion

On the basis of our results we have found that the sensitivity of cyanobacteria species were greater in thylakoidal fragments than spheroplast. When thylakoidal fragments were immobilized on working electrodes gave better results than spheroplast and intact cells. We observed four types of major peaks in UV-Vis spectroscopy, one peak of chlorophyll a in red light region (680 nm) and trace of chlorophyll a in blue light region (440 nm), one peak of carotenoid (490 nm) and one peak of phycobilisomes (650 nm in our case) which shifted in the presence of herbicides as they affected PSII. We also have found that at certain concentration of 2, 4 D (10-4 M), growth was promoted instead of showing inhibitory effect. The electrode with immobilized samples may be kept at -20°C for a minimum time of 12 h.

Acknowledgement

The authors are grateful to the CSIR and Maulana Azad Scheme for providing fellowships.

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