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Lead Induced Alterations in PSII and Lipid Peroxidation of *Oscillatoria subbrevis* and its Relevance in Bioremediation of Lead

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Abstract

In this present investigation, we have made a study on alterations in photosystem II electron transport activity and lipid peroxidation at different concentrations of lead [$Pb(NO_3)_2$] (5, 10 and 15 µM) in the thylakoid membranes of *Oscillatoria subbrevis.* When the concentration of lead is increased from 5 µM to 15 µM, it gradually caused enhancement in the lipid peroxidation from 34 n moles up to 43 n moles concentration. At 15 µM Pb concentration in the medium 43% of enhancement in lipid peroxidation was noticed. This heavy metal (Pb) induced lipid peroxidation is responsible for the damage of PS II and its related electron transport activity. An investigation was done to know the protective action of ascorbate to minimize the lipid peroxidation, by addition of 8µM concentration of ascorbate in the medium with 15µM concentration of lead heavy metal. The results has shown that Ascorbate acts as a protective agent to minimize lipid peroxidation and enhance photosynthetic activity which will help in the better survival of these cyanobacterial species and their promising use in the bioremediation of industrial effluents.

Keywords: Bioremediation; Cyanobacteria; Lipid peroxidation; Electron transport activities.

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1. Introduction

Bioremediation has emerged as the most desirable technology which uses cyanobacterial species and plants for removal of environmental pollutants or detoxification to make them harmless [1]. There are various cyanobacteria used in the bioremediation has a considerable capacity of metal absorption, its accumulation and reducing the time of decontamination of an ecosystem. As the industrial waste waters are mostly discharged into the water bodies we are more concerned about the effect of those heavy metals on the aquatic flora. There are various experiments and reports on the basis of which we can think of freshwater cyanobacteria to be used as agents in bioremediation process [2]). The cyanobacteria have many features that make them ideal for the selective removal and reducing the concentration of heavy metals, which include high absorption capacity, high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/ volume ratios, phototaxy, phytochelation production and its potential for genetic manipulation [3].

There are several reports that different species of several fresh water microalgae like *Chlorella sp.*, *Anabaena sp.*, *Westiellopsis sp.*, *Stigeoclonium sp.*, *Synecococcus sp.* etc. have high tolerant capacity for various heavy metals [4]. The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and functional groups on the surface or inside the porous structure of the biological material. Metals are taken up by algae through adsorption over the cell surface very quickly which is called as physical adsorption and then these ions are transported slowly into the cytoplasm through the process called chemisorption. When living *Spirullina* cells were used to remove lead from the aqueous medium containing 50mg/L lead, it showed that initially the adsorption rate was rapid i.e., 74% of metal was biologically adsorbed [5].The adsorption, phytosorption and affinity of Cyanobacteria is high for these metal cations because of their high negatively charged cell wall components. Ionic strength of the media also plays an important role on metal ion uptake; decrease in ionic strength helps increase in removal efficiency of metal ions from the media. It was reported that when ionic strength decreased the removal efficiency is increased which may be due to the competition for the functional groups between the metal ions and other ions which played an important role [6]. Cyanobacteria are grouped in the phylum of bacteria; generate energy by the process of photosynthesis. This process is very flexible with molecular machinery and biochemical-mechanisms. The group of cyanobacteria contribute nearly 40% for production of biomass globally by using the solar energy into biomass-stored chemical energy [7]. The heavy metals are toxic and found to disturb the photosynthetic process in these cynobacterial species. Heavy metal are grouped into essential metals (Fe, Zn and Cu) and nonessential metals (Cd, Pb, Hg, As).The essential metals help in numerous physiological processes and photosynthetic reactions, but at higher elevated concentrations they also cause toxic impairment in the whole photosynthetic system. Even at low concentrations the nonessential heavy metals adversely affect the productivity of cynobacterial species whose biological functions are not clear [8].

2. Materials and Methods

2.1. Organism

The mother cultures were obtained from Department of Microbiology, National Facility for Marine

Cyanobacteria, Bharathidasan University, Tiruchirappalli, India and cultured autotrophically in BG-11 medium [9]. The thylakoid membranes are prepared by following the procedure of Katoh (1988)

2.2. Heavy metal treatment

Cells from the late log grown cultures were harvested by centrifuging at 10,000 X g for 5 min, washed once with the same growth medium and finally suspended in the same medium. The collected cells were suspended in 25mM HEPES-NaOH buffer (pH 8) at a Chl conc of 200 µg mL^{-1}. Samples were incubated by using $Pb(No_3)_2$ at the concentration of (5, 10 and 15µM) concentrations for a period of 12-60 hrs and polarographic measurements are done according to Murthy, 1991 with minor modifications. Parabenzoquinone (pBQ) was used to measure the PS II catalyzed electron transport ($H_2O \rightarrow pBQ$) in the intact cells. Being a lipophilic compound pBQ enters into the intact cells and accepts electrons at plastoquinone (PQ) position [12,13]. The reaction mixture contained reaction buffer (same as used in cell).

2.3. Lipid peroxidation

Lipid peroxidation (LPO) has been measured in terms of production of malondialdehyde according to the method of Heath and Packer [1968] with minor modifications. The malondialdehyde (MDA) calculations were made by using the extinction coefficient 155 mM^{-1} cm^{-1}. The amount of MDA was expressed as n M of MDA per mg protein.

3. Results and Discussion

Thylakoid membranes are rich in lipids, particularly monogalactosyl diacyl glycerol (MGDG) and digalactosyl diacyl glycerol (DGDG) for about 60-70 % of the membrane lipids [15]. Studies on the mixtures of MGDG and DGDG suggest that the increase in the heavy metal favours the formation of non-lamellar structures. The non-bilayer lipids are required for the package of light harvesting units of PSI and PS II in to thylakoid membranes. Thylakoid membranes contain proteins in addition to lipid bilayer [16]. The major proteins present in thylakoid membranes are polypeptides related to WOC are LHC, and polypeptides related to intersystem electron carriers (Cyt b_6f), and polypeptides related to PS I and PS II. The changes under stress in the membrane organization can affect the functional aspects of photosynthetic electron transport chain besides PS II. Therefore an attempt was made to study the effect of Pb metal stress on thylakoid membrane organization in this cyanobacterium by increasing the concentration from 5 to 15µM. The cells were grown in media containing $Pb(NO_3)_2$ of concentration of 5-15 µM for 60 hrs and lipid peroxidation was measured in terms of MDA formed. In control cells 30 n mole *MDA/mg* proteins was observed. When the concentration of lead increased from 5-15 µM gradually caused enhancement in the lipid peroxidation up to from 34- 43 n mole concentration. At 15 µM Pb concentration in the medium 43% enhancement was noticed.

Meenakshi Chaudhary and their team in [17] reported the effect of heavy metals stress on malondialdehyde (MDA) activity in the cyanobacterium: *Spirulina platensis* S5. Their work clearly demonstrated the toxic impact of lead, copper, zinc over a concentration gradient of (0.05-0.20 mg/L on malondialdehyde (MDA). Increase amount of MDA was indicative of formation of free radicals in the test microorganisms under heavy metal

stress. The findings are supported by the Gupta and their co-workers [18] by publishing the review article "Tolerance against heavy metal toxicity in cynobacteria: Role of antioxidant defence system".

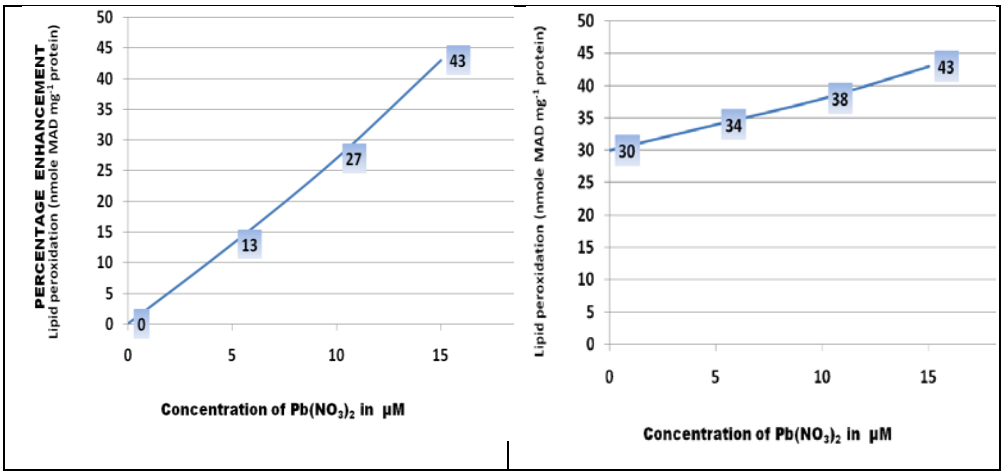

Figure 1: Effect of different concentrations of lipid peroxidation of thylokoid membranes

An attempt was made to study the comparative effect of Pb metal stress on PS II catalyzed electron transport in relation with lipid peroxidation of thylakoid membranes. For this study the cells were grown under different lead concentrations in the medium and in the same samples both PS II electron transport activity as well as lipid peroxidation was measured. The increase in concentration gradually caused inhibition in the PS II activity. The inhibition of PS II activity is very much related with the increase of lipid peroxidation. In this way there was an inverse relationship between electron transport activity and lipid peroxidation in treated samples.

Table 1: Effect of Pb on PS II catalysed electron transport and lipid peroxidation:A comparative study in thylakoid membranes of *Oscillatoria subbervis.*

Concentration $Pb(No_3)_2$(µM)	**PS II activity µmol O_2 evolved $mg(Chl\text{-}^{1})\ h^{-1}$**	**Lipidperoxidation $nmol(MDA)mg^{-1}$ (protein)**
control	323 ± 21	30 ± 3.1
5	280 ± 14	34 ± 5.0
10	237 ± 12	38 ± 4.1
15	176 ± 10	43 ± 4.9

To analyse the possible scavenging mechanism (protection) of lipid peroxides a study has been made using ascorbate (8 µM) as a protectant. For this study, cells were incubated at room temperature for 45 min in 15µM concentration of lead and both in the presence and absence of ascorbic acid. In the absence of ascorbate 43% increase in lipid peroxidation was noticed. But when the cells were incubated with 8µm concentration of

ascorbate in the reaction mixture even at room temperature and in presence of lead (15µM), almost the lipid peroxidation was drastically suppressed. In the presence of ascorbate the lipid peroxidation even at these high concentrations of heavy metal is almost equal to that of control cells. Thus ascorbate at 8µM concentration protects the peroxidation of lipids in heavy metal treated cells of *O.subbrevis*. This result helps in the protection of this cyanobacterium *O.subbrevis* and also helps in the good performance of primary process of photosynthesis which inturn increases the survival of the organism. This level of alteration in the bacterial growth depends on the intensity, duration of stress and its combination with others. These different stresses influence the bacterial growth and other physiological activities of photosynthesis.

Table 2: Ascorbate (8 µ M) mediated protection of thylakoid membrane lipid peroxidation under Pb metal stress

$Pb(N0_3)_2$	**Lipid peroxidation (n mol MDA mg^{-1}protein)**	**Percentage increase**
Control	30±3.1	0
15µM $Pb(N0_3)_2$ (Absence of Ascorbate)	43±5.8	43
15µM$Pb(N0_3)_2$(Presence of (8µM)Ascorbate)	30±2.2	0

4. Conclusion

The cyanobacterial species are very much helpful in the bioremediation process if we enhance their survival capacity in the presence of heavy metals.

The cyanobacterial species can act as a promising biological tool and bio-marker in reducing impact of heavy metals in the industrial effluents if we protect this cyanobacterium: ***Oscillitoria subbrevis***.

The heavy metal (Pb) concentrations enhanced lipid peroxidation is responsible for the damage of PS II. Enhanced Lipid peroxidation by the action of 15µM concentration of lead heavy metal can be minimized by the addition of 8µM concentration of ascorbate in the medium.

Ascorbate acts as a possible protection mechanism to minimize lipid peroxidation which will help in the better survivalist character of these cyanobacterial species.

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