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Heavy Metal Tolerance Studies of Cyanobacterial Species Under Experimental Conditions

Abstract

Water inhabiting autotrophic microorganisms has the ability to grow in mass quantity under ideal conditions and such ability provides usable biomass at minimal efforts. These microorganisms are reported to play a significant role in heavy metal absorption from contaminated water in which they are growing. In the present study two Cyanobacterial isolates were cultured using different growth media (nitrogen plus and nitrogen free). The isolates were studied for their ability to tolerate heavy metals under experimental conditions by the dilution method with different concentrations (5, 10, 15ppm). Among the heavy metals Cu, As, Pb and Hg are reported to require for correct functioning of living organisms but an excess or deficiency of these metals can promote severe toxic effects, which depends on the type of organism, the nature and concentration of the metal and environmental conditions. Based on above assumption both *Synchocystis sp.* and *Oscillatoria sp.* showed various degree of tolerance for selected heavy metals.

Keywords: Heavy Metals, Toxicity, Tolerance, Cyanobacteria, Growth

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Introduction

Cyanobacteria are the largest and most diverse group of photosynthetic prokaryotes. Their habitats vary from fresh and marine water to terrestrial environment. Heavy metals have been released to the environment over long periods of time by natural process and manmade activities generate their anomalous concentration. These metals are directly or indirectly involved in all phases of microbial growth and enter in to higher levels of food chains. All these metals can be toxic to biotic components and in some cases the beneficial to extremely narrow ranges.

Many cyanobacterial sp. are reported remarkable affinity for heavy metals (Gale and Wixson, 1979 and Audholia *et al.* 1993), these cells have developed natural methods of responding to metals such as copper, lead, cadmium, arsenic and mercury through passive accumulation in cells and through surface binding to various functional groups. They have also been found to remove harmful metals from the environment.

In the present study, two abundantly growing indigenous Cyanobacteria species were studied under experimental conditions to obtain large biomass and their comparative tolerance for some heavy metals.

Material and Methods

Isolation and Selection of best Growth medium – Out of all the isolates, from different water bodies of Durg Rajnandgaon area (Chhattisgarh) two strains viz. *Synchocystis sp*. and *Oscillatoria sp* was found to have higher ability to grow in mass quantity under culture conditions. Monocultures of *Synchocystis sp*. and *Oscillatoria sp*. were developed by isolation and identified by the monographs provided by Desikacharya (1959) and Presscott (1962). The isolates were grown on different growth medium such as Allen's and Arnon's broth, BG-11 broth, Chu 10 broth, Pringschim's broth (nitrogen plus and nitrogen free) axenically at 28±2ºC temperature with continuous illumination of intensities of 2000 Lux. Growth were compared by OD_{550} and recovery of dry biomass (Yoshida *et al*. 2005).

Isolation of Heavy Metal Tolerance

The monocultures of *Synchocystis sp*. and *Oscillatoria sp*. were screened for isolation of heavy metal tolerance under experimental conditions. Sterilized solution of different heavy metals Arsenic Sulphite (As), Cuprous chloride (Cu), Mercorous oxide (Hg), and Lead carbonate

(Pb) at the rate of 5, 10, 15ppm individually, were aseptically supplemented with suitable solid culture medium. Tolerant colonies were selected and re-cultured for two more times and finally transferred to heavy metal containing broth. Growth of individual monoculture was evaluated by OD_{550} and recovery of dry biomass and compared with control (Yoshida *et al*. 2005).

Analysis of Data

Five replicates were maintained throughout the studies and data were analyzed by suitable statistical methods (Muthukumar *et al*. 2007).

Result and Discussion

The monocultures were grown on different growth medium individually and observed after 30 days for growth in terms of OD_{55O} and recovery of Dry biomass (Table -1-2). BG 11 (nitrogen free) media and Pringhschim's broth (nitrogen free) media were found as best suitable media for *Synchocystis sp*. and *Oscillatoria sp*. respectively in terms of OD_{55O} and Dry biomass. The selected media was used further for heavy metal tolerance experiment.

The effect of Arsenic Sulphide (Table - 3) was found more tolerable by *Oscillatoria sp*. as compared to *Synchocystis sp*. however, both the organisms responded well in terms of OD_{55O} and Dry biomass up to 10 days of incubation and up to 15ppm concentration.

The Mercurous Oxide also responded higher in terms of biomass (Table - 4) for both of the test cyanobacteria in 5ppm concentration. The higher concentration however, resulted in gradual decrease of growth up to 15ppm concentration and 15days of incubation respectively.

Effect of Lead tolerance is depicted in Table - 5 reveals that Lead Carbonate affected well up to 10days of incubation in 5 and 10ppm concentration. There was a decrease in growth under 10ppm concentration and 15days of incubation respectively.

Screening for copper tolerance (Table - 6) reveals that Cuprous Chloride is also affected well up to 10ppm for both of the test cyanobacterial sp. The higher concentration and larger incubation days gradually resulted in decrease in the growth as compared to control (Table - 2).

Among the heavy metals Cu, As, Pb and Hg are reported to require for correct functioning of living organisms but an excess or deficiency of these metals can promote severe toxic effects, which depends on the type of organism, the nature and concentration of the metal and environmental conditions. This study was focused on the effects of heavy metals on cyanobacterial species in terms of OD_{550} and Dry biomass. Mann *et al.,* (2002) studied the case of Cu, even the picometer concentrations that occur in the oceans seem to be toxic to some cyanobacteria. According to Kowalewsla *et al.,* (1992) the beneficial range of Cu concentration is very narrow, its needed as an active centre in many proteins and enzymes, but even it is still toxic to some cyanobacteria. Kupper and Kroneck also studied the heavy metal uptake by plants and cyanobacteria. In adsorption studies on heavy metals by Kumaran *et al.* the isolated cyanobacteria (*Nostoc sp*.) were accumulated more than 91% of the heavy metals from the estuarine water.

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Table - 1 Selection of Best Growth Media

S.No.	Growth Media	*Synchocystis sp.*				*Oscillatoria sp.*			
		Od_{500nm}		Dry Weight (g)		OD_{500nm}		Dry Weight (g)	
		$+N_2$	$-N_2$	$+N_2$	$-N_2$	$+N_2$	$-N_2$	$+N_2$	$-N_2$
1	Allens and Arnons	0.57	0.60	0.95	1.00	0.04	0.05	1.00	0.62
2	BG-11	0.58	0.63	0.97	1.02	0.07	0.09	1.07	0.60
3	Chu#10	0.00	0.18	0.00	1.05	0.09	0.10	0.64	0.80
4	Pringhschims	0.00	0.64	0.00	1.01	0.06	0.12	0.59	0.85

Table -2 Growth Studies of *Synchocystis Sp*. and *Oscillatoria Sp.* in Different Incubation Periods

S.No.	Incubation period (Days)	*Synchocystis sp*. BG 11(-N_2)		*Oscillatoria sp.* Pringhschims broth (-N_2)	
		OD_{550}	Dry weight* (g)	OD_{550}	Dry weight* (g)
1	5	0.60	1.02	0.11	0.85
2	10	0.62		0.16	
3	15	0.63		0.19	

*after 15 days of incubation

Table - 3 Screening Of *Synchocystis Sp*. And *Oscillatoria* Sp For Arsenic Tolerance.

S.No.	Concentration of Heavy Metal	*Synchocystis sp*. BG 11(-N_2) + Arsenic sulphite				*Oscillatoria sp.* Pringhschims broth (-N_2) +Arsenic sulphite			
		OD_{550}			Dry weight* (g)	OD_{550}			Dry weight* (g)
		I	II	III		I	II	III	
1	5 ppm	0.967	0.810	0.372	1.06	1.137	0.979	0.391	1.10
2	10ppm	1.081	0.796	0.298	1.02	1.161	0.887	0.406	1.05
3	15ppm	1.017	0.791	0.243	1.00	1.155	0.819	0.360	1.01

I,II,III – 5,10, and 15 days of incubation
*after 15 days of incubation

Table – 4 Screening of *Synchocystis Sp*. and *Oscillatoria* Sp for Mercury Tolerance

S.No.	Concentration of Heavy Metal	*Synchocystis sp*. BG 11(-N_2) + Mercorous oxide				Oscillatoria sp.Pringhschims broth (-N_2) + Mercorous oxide			
		OD_{550}			Dry weight* (g)	OD_{550}			Dry weight* (g)
		I	II	III		I	II	III	
1	5 ppm	1.060	0.959	0.569	1.26	0.783	0.796	1.00	1.14
2	10ppm	0.987	0.689	0.345	0.93	0.658	0.599	0.321	1.11
3	15ppm	0.991	0.639	0.301	1.03	0.688	0.639	0.317	1.08

I,II,III – 5,10, and 15 days of incubation
*after 15 days of incubation

Table – 5 Screening of *Synchocystis Sp.* and *Oscillatoria* Sp for Lead Tolerance

S.No.	Concentration of Heavy Metal	*Synchocystis sp*. BG 11(-N_2) + Lead carbonate				*Oscillatoria sp.* Pringhschims broth (-N_2) + Lead carbonate			
		OD_{550}			Dry weight*(g)	OD_{550}			Dry weight*(g)
		I	II	III		I	II	III	
1	5 ppm	1.149	0.695	0.418	1.17	1.056	0.887	0.346	1.17
2	10ppm	1.046	0.688	0.318	0.96	1.051	0.796	0.459	0.83
3	15ppm	1.040	0.639	0.278	0.98	1.012	0.695	0.305	0.68

I,II,III – 5,10, and 15 days of incubation
*after 15 days of incubation

Table – 6 Screening of *Synchocystis Sp*. and *Oscillatoria* SpfFor Copper Tolerance

S.No.	Concentration of Heavy Metal	*Synchocystis sp*. BG 11(-N_2) + Cuprous chloride				*Oscillatoria sp.*Pringhschims broth (-N_2) + Cuprous chloride			
		OD_{550}			Dry weight*(g)	OD_{550}			Dry weight*(g)
		I	II	III		I	II	III	
1	5 ppm	1.086	0.689	0.441	1.01	1.187	0.887	0.389	1.14
2	10ppm	1.086	0.639	0.359	1.03	1.161	0.796	0.393	1.09
3	15ppm	0.987	0.599	0.276	1.04	0.724	0.639	0.165	1.13

I,II,III – 5,10, and 15 days of incubation
*after 15 days of incubation