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INFLUENCE OF SOME METALS ON GROWTH OF TWO ANOXYGENIC PHOTOTROPHIC BACTERIA

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ABSTRACT

The presence of photosynthetic bacteria along with the heterotrophic bacteria has been reported in various aquatic environments like Indian tropical waters, salt marshes, industrial effluents, seawater, sewage water and hot water springs. Two anoxygenic phototrophic bacteria ***Rb. capsulatus*** and ***Rps. acidophila*** were isolated from leather industrial effluents and influence of some heavy metals on the growth of these two bacteria was studied. Iron and cadmium requirement for the growth of ***Rb. capsulatus*** and ***Rps. acidophila*** was very low, while selenium and cerium failed to influence the growth of both the bacteria under investigation. ***Rps. acidophila*** was highly sensitive to and was totally inhibited by mercuric chloride at a very low concentration, whereas ***Rb. capsulatus*** exhibited considerable resistance. Biological significance of above observations in the light of existing literature is discussed.

INTRODUCTION

Heavy metals constitute a heterogeneous group of elements widely varied in their chemical properties and biological functions. Heavy metals are kept under environmental pollutant category due to their toxic effects on plants, animals and human beings. Natural waters have been found to be contaminated with several heavy metals arising mostly from mining wastes and industrial discharges (Schalcsha & Ahumada 1998, Grousset et al. 1999). Unlike organic contaminants, which can be degraded to harmless chemical species, heavy metals cannot be destroyed. The effects of metals on functioning of ecosystems vary considerably and have economic and public health significance. The main sources of metal ions in streams are effluents from industries, such as electroplating, paints, plastics and battery. Acute lead, cadmium, chromium and copper poisoning in humans causes severe dysfunction in the renal, reproductive and nervous systems (Berman 1980, Yong et al. 1998). Besides, chronic exposure to these contaminants, present even at low concentrations in the environment, can prove to be harmful to the human health (Wyatt et al. 1998). The use of microbial cells as biosorbents for heavy metals offers a potentially inexpensive alternative compared to conventional methods of heavy metal decontamination from a variety of industrial aqueous process streams (Gomes et al. 1998, Knorr et al. 1991, Khoo & Ting 2001).

Many members of Rhodospirillaceae show a high resistance towards toxic heavy metal oxides and oxyanions (Moore & Kaplan 1992). ***Rhodobacter sphaeroides*** and ***Rhodovulvum*** spp. were capable of cadmium removal in a batch culture system. Removal of phosphorus from oyster farm mud sediment using a photosynthetic bacterium, ***Rhodobacter sphaeroides*** IL 106 was studied by Takeno et al. (1999). ***Rhodobacter sphaeroides*** grew in the presence of up to 43 mM chromate and reduced hexavalent chromium to the trivalent form under both aerobic and anaerobic conditions (Nepple et al. 2000). Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium ***Rsp. rubrum*** was studied by Kessi et al. (1999). Microbial growth was considerably reduced when heavy metal concentration was higher than 1 mg/L. Besides, ***R. sphaeroides*** was quite tolerant to copper. Cultures to which copper was added from 0.01 to 10 mg/L showed a relatively constant growth, both in aerobic and anaerobic conditions (Balsalobre et al. 2006). ***Rhodobacter sphaeroides*** was proved to be highly tolerant to heavy metal exposure, especially towards Co2+, Fe2+ and MoO_2^{-4}. In addition Ni^{2+} and Co^{2+} were found to decrease the cellular content of the light harvesting complexes (Giotta et al. 2006). A fundamental study of the application of bacteria to the recovery of toxic heavy metals from aqueous environments was carried out. The biosorption characteristics of cadmium and lead ions were determined with purple nonsulfur bacteria, ***Rhodobacter sphaeroides*** and hydrogen bacteria, *Alcaligenes eutrophus* H16 which were inactivated by steam sterilization (Seki et al. 1998). Assessment of heavy metal toxicity using bacterial tests, when grown in different environments, was reported by Balsalobre et al. (2006). In this paper the ability of the microorganisms to cope with metal pollutants is presented.

MATERIALS AND METHODS

The data on the influence of the metals on growth of the two anoxygenic phototrophic bacteria are given in Table 1. Phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the BP medium and incubated anaerobically in the light. The cultures obtained by enrichment technique were streaked onto the solid medium repeatedly and colonies were picked up to inoculate into the liquid medium and maintained by subculturing. Bacteria, thus, isolated were identified by studying the cultural characteristics (colour, size and shape), utilization of carbon and nitrogen sources, vitamin requirements, absorption spectral analysis, bacteriochlorophyll and carotenoids with the help of Bergey's Manual of Systematic Bacteriology.

Pure cultures of the two anoxygenic phototrophic bacteria were maintained in Beibl and Pfennig's medium (BPM) containing mg/L medium of $MgSO_4.7H_2O$:200; NaCl:400; NH_4Cl:400; $CaCl_2.2H_2O$:50; succinate:1000; yeast extract:200; ferric citrate solution (0.01g/mL, 1mL), and the trace element solution 1mL and cyanocobalamine (vitamin B_{12}, 0.01g/L, 1mL). Various concentrations of the said metal were added into the medium. Fifteen mL screw cap tubes, filled with medium and inoculated with 1mL (5days old cultures) of one of the two anoxygenic phototrophic bacteria, were incubated for 8 days at 30+2°C under the light intensity of 2000 Lux. The experiments were run in triplicate. The growth of the bacteria was determined with the help of spectrophotometer at 660nm with uninoculated medium as blank. Final pH of the medium was recorded with the help of a pH meter.

RESULTS AND DISCUSSION

Rps. acidophila was highly sensitive and totally inhibited by mercuric chloride at 0.03 mM concentration, while ***Rb. capsulatus*** was inhibited at 60 mM, which increased with the increase in concentration of mercuric chloride. Interestingly pH of the media did not change much both with the growth and concentration of mercuric chloride. Potassium dichromate was also toxic to ***Rps. acidophila*** and it was responsible for total inhibition of growth at 0.85 mM concentration. ***Rb. capsulatus*** was comparatively more resistant as it could grow even at 0.80 mM concentration. $HgCl_2$ did not permit the growth of ***Rps. acidophila***, while ***Rb. capsulatus*** exhibited considerable resistance as it could make significant growth even at 0.036 concentration of $HgCl_2$ even though at 0.003 mM concentration the growth inhibition was more than 50%. Cadmium was toxic to both the bacteria under investigation. The toxicity of metal increased with the increase in its concentration. Cerium sulphate was found to be toxic and its toxicity increased with the increase in the concentration of cerium. Selenium was

Table1: Influence of some metals on the growth of two anoxygenic phototrophic bacteria.

Heavy metal	Concentration (mM)	*Rb. capsulatus*		*Rps. acidophila*	
		Growth (in O.D)	pH	Growth (in O.D)	pH
Ferric citrate	0	1.084	6.8	0.915	5.8
	0.03	1.056	7.0	0.892	6.0
	0.07	0.954	7.0	0.814	6.2
	0.11	0.803	7.0	0.775	6.2
	0.15	0.724	7.0	0.662	6.2
	0.19	0.663	7.2	0.567	6.2
	0.23	0.645	7.2	0.545	6.2
Selenium dioxide	0	1.084	6.8	0.915	5.8
	0.22	0.912	7.0	0.862	6.0
	0.45	0.883	7.0	0.725	6.0
	0.9	0.864	7.0	0.684	6.0
	1.3	0.812	7.2	0.653	6.2
	1.8	0.754	7.2	0.624	6.2
	2.8	0.612	7.2	0.594	6.2
Ceric sulfate	0	1.084	6.8	0.915	5.8
	0.06	0.624	7.0	0.693	6.0
	0.12	0.452	7.0	0.522	6.0
	0.24	0.413	7.2	0.508	6.0
	0.37	0.406	7.2	0.463	6.0
	0.5	0.382	7.2	0.312	6.2
	0.6	0.337	7.2	0.298	6.2
Cadmium chloride	0	1.084	6.8	0.915	5.8
	0.09	0.687	7.0	0.622	6.0
	0.18	0.523	7.0	0.593	6.0
	0.375	0.486	7.0	0.578	6.0
	0.563	0.454	7.2	0.524	6.2
	0.75	0.362	7.2	0.484	6.2
	0.93	0.327	7.2	0.422	6.2
Potassium dichromate	0	1.084	6.8	0.915	5.8
	0.08	0.523	7.0	0.534	6.0
	0.17	0.384	7.0	0.305	6.2
	0.34	0.354	7.0	0.278	6.2
	0.51	0.308	7.2	0.212	6.2
	0.68	0.286	7.2	0.196	6.2
	0.85	0.223	7.2	-	6.4
Mercuric chloride	0	1.084	6.8	0.915	5.8
	0.003	0.452	7.2	-	6.0
	0.007	0.332	7.2	-	6.0
	0.01	0.316	7.2	-	6.0
	0.02	0.294	7.2	-	6.0
	0.029	0.288	7.2	-	6.0
	0.036	0.266	7.2	-	6.0

comparatively mild toxic element as the degree of inhibition increased only marginally with the increase in concentration of selenium. Similarly iron turned out to be toxic above 0.11 mM concentration and the degree of inhibition increased only marginally with the increase of iron concentration.

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