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Chromate Reduction by a Purple Non Sulphur Phototrophic Bacterium *Rhodobacter capsulatus* KU002 Isolated from Tannery Effluents

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An extensive and intensive survey of sewage, industrial effluents, rice fields, freshwater and earthworm casts for the presence of purple non-sulphur bacteria was undertaken from Warangal district of Andhra Pradesh state in India. In all the nine bacterial species, which included *Rhodopseudomonas palustris, R.rutila, R.acidophila, Rhodopila globiformis, Rhodospirillum rubrum, Rsp.photometricum, Rhodobacter sphaeroides, Rb.capsulatus, Rhodobacter* sp and *Rhodocyclus gelatinosus* were isolated. Among these *Rhodobacter capsulatus*, isolated from leather industry effluents could reduce maximum amount of chromium and was selected for further studies. Effect of alginate immobilization on reduction of chromium by *Rhodobacter capsulatus* was also investigated. *Rb.capsulatus* could reduce about 28 μM of chromium. Chromate reduction by free cells was less and was only 19 μM. Maximum reduction of chromium by free or immobilized cells of *Rb. capsulatus* took place on 12th day itself. Optimal activity of reductase was shown to take place at pH 7.6 and a temperature of 38°C. Enzyme activity was found to be in soluble fraction. Significance of the above results in light of existing literature is discussed in this communication.

Key words: *Rhodobacter capsulatus,* Chromate reduction, Anaerobic light, Chromate reductase.

Heavy metals are the main groups of inorganic contaminants and a considerable large areas of land is contaminated with them due to use of sludge or municipal compost, pesticides, fertilisers and emission from municipal compost, incinerators, car exhaust, residues from metalliferous mines and smelting industries. Chromium is the seventh most abundant metal in the earths crust. Hexavalent chromium is capable of penetrating the membrane into cytoplasm, where it is reduced to trivalent and reacts with the intracellular material (Halim *et al*.,2003). Biological transformation of Cr(VI) to Cr(III) by enzymatic reduction provides a less costly and more environmentally friendly approach to remediation. Cr(VI) reduction has been demonstrated in various bacterial species including *Bacillus* sp.(Liu *et al*.,2006), *Pseudomonas* sp.(Ishibashi *et al*.,2006; Park *et al*.,2000), *Escherichia coli* (Bae *et al*.,2001), *Desulfovibrio* sp.(Mabbett and Macaksie,2001), *Arthrobacter* sp.(Asatiani *et al*.,2004) and *Rhodobacter* sphaeroides (Nepple *et al*.,2000). Anoxygenic phototrophic bacteria are the most widely used organisms for bioremediation purposes. Except the reports of Nepple *et al*. (2000, 2002) and Giotta *et*

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al.(2006) no information is available on chromate reduction by Purple non sulphur bacteria. This is the first study on chromate reduction by *Rhodobacter capsulatus* which was isolated from leather industry effluents.

The phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the medium and incubated anaerobically in the light (2000 lux). Bacteria thus isolated were identified with the help of cultural characteristics (colour, size and shape), carbon and nitrogen requirement, vitamin requirements, absorption spectra analysis, bacteriochlorophylls and carotenoids. Identification keys provided in Bergey's manual of systematic bacteriology (1994) was adopted.

Bacteria were separated by centrifugation at 5000 rpm at 4°C for 10 min. The cells were washed and resuspended in sterile saline. The alginate entrapments of cells were performed according to the method of Johnsen and Flink (1986). Chromium was assayed by the Diphenyl carbazide method (Greenberg *et al*.,1992) Chromate reductase activity was determined by measuring the decrease in chromate concentration during enzyme assays. The optimal pH of the reductase activity was determined using the buffers citric acid-NaOH (pH 3 to 6), sodium phosphate (pH 6 to 7), and Tris-HCl (pH 7 to 9). The enzyme heat stability was determined by exposing it to different temperatures. The enzyme was then cooled on ice and assayed for residual activity. The incubation time for determining the optimal pH and temperature was 10 min. To find out which fraction contained the reductase activity, the cells were disrupted by using a sonicator. The lysate was centrifuged (10,000 rpm for 20 min) to remove unbroken cells and debris to get the soluble fraction for reductase activity.

Perusal of table 1 shows that *Rb. capsulatus* could reduce about 28 mM of chromium. Chromate reduction by free cells was less and was only 19 mM. Immobilized cells could not reduce anymore chromium after the 12th day. Although there was a slight increase in chromium reduction by the free cells on the 16th day, it was

Table 1. Growth , pH changes and reduction of chromate by *Rb.capsulatus*

Organism	Incubation (in days)	Growthin O.D	Final pH	Free cells (µM)	Immobilized cells (µM)
Rb.capsulatus	4	0.866	7.0	12	18
	8	1.115	7.4	16	24
	12	0.956	8	18	28
	16	0.728	8.2	19	28

negligent. Maximum reduction of chromium by free or immobilized cells of *Rb. capsulatus* took place on12th day itself. pH changes were towards the alkaline side. Chromate reduction by some phototropic bacteria upto 20 µM was reported by Nepple *et al*. (2000) without immobilization. Optimal chromate reduction was related to the growth of the bacteria under investigation. Extreme pH restricted bacterial growth and chromate reduction. Chromate reduction could be carried out for prolonged periods of time using phototrophic bacteria. Chromate reduction could be seen from pH 4.5 onwards to pH 8.0. Optimal chromate reduction was not related to the growth of the bacteria under investigation. Studies on optimal activity of reductase showed the enzyme was active at pH 7.6 in Tris-Hcl buffer and required a temperature of 38°C. Enzyme activity ceased at 50°C. Chromate reductase activity was present exclusively in the soluble extract. The enzyme was not inducible and was present irrespective of the presence of chromium in the medium. Chromate reductases from *P.putida* MK1, *P. putida* PRS2000 (Park *et al*.,2000) and *P. ambigua* (Suzuki *et al*.,1992) were also found to be in the soluble extract. Our observations are in agreement with that of Nepple *et al*.(2002) who also reported the presence of reductase activity in the soluble

fraction. Further exploration and exploitation of this group of bacteria may be taken up as they show considerable resistance to various metal ions.

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