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Study on the Detoxification of Chromium by Microorganisms Isolated from Tannery Effluent

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Three bacterial isolates and one fungal isolate were obtained from a local tannery effluent. The isolates were identified and characterized based on their morphological and biochemical characters. Of the isolates obtained, *Staphylococcus aureus* was found to be the most efficient in detoxification of hexavalent chromium, the most toxic form of chromium in effluents. Comparative study and effect of chromium on pigment production was also studied.

Key Words: Hexavalent chromium, *Staphylococcus aureus*, Detoxification.

Tannery effluent is a major source of aquatic pollution in India with high chemical oxygen demand (COD), biological oxygen demand (BOD) and hexavalent chromium [Cr(VI)]. There are a large number of tanneries scattered all over the country but the main areas they are concentrated are Tamil Nadu, Uttar Pradesh and West Bengal (Sumit Yadav *et al*., 2005). The tanning industry discharges different types of wastes into environment, primarily in the form of liquid effluents containing organic matters, chromium, sulphide, ammonium and other salts (Indhu Shekar Thakur, 2006). Pollution becomes acute when tanneries are concentrated in clusters in arid areas, as in the case of Tamil Nadu.

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Chromium, a steel gray, hard and brittle metal occurs in nature in bound forms that constitute 0.1-0.3mg/kg of the earth's crust. It has several oxidation states ranging from Cr(-II) to Cr(+VI), where the trivalent and hexavalent states are the most stable ones (Adel M Zayed, 2003). In trace amounts, chromium is considered as an essential nutrient for numerous organisms, but at higher level, it is toxic and mutagenic (Shen *et al*., 1993).

Camargo *et al*, 2003 reported chromium-resistant bacteria from soils contaminated with dichromate. Most isolates tolerated and reduced Cr (VI) at concentrations lower than 1500mg/l chromate belonged to genus *Bacillus*. A number of chromium resistant microorganisms have been reported, including *Serratia marcescens* (Mondaca *et al*, 2002), *Microbacterium* (Pattanapipitpaisal *et al*., 2001), *Enterobacter sp.* (Wang *et al*., 1990), *Escherichia coli* (Shen *et al*., 1993), *Shewanella alga* (Guha *et al*., 2001), *Bacillus sp* (Campos *et al*., 1995). The present study was undertaken to find novel organisms from a local tannery effluents that could detoxify the toxic hexavalent chromium to non-toxic trivalent chromium.

MATERIALS AND METHODS

Sample collection

Polythene containers were used for the collection of effluent from tannery industry in Pallavaram, Chennai. The samples were labeled and transported to the lab.

Screening for bacteria

A loopful of the effluent sample was inoculated on plates containing nutrient agar or in chocolate agar for growing fastidious bacteria. The bacterial isolates were characterized by using staining methods (simple staining, gram staining), and conventional biochemical tests and the results tabulated. The isolated pure cultures were subcultured and maintained.

Screening for fungi

PDA was inoculated using a sterile cotton swab dipped in the effluent. The plates were incubated at 37°C for 72 hours to 1 week.

Detoxification studies

The cultures were activated by culturing them in Vogel-Bonner Broth (VB Broth) which is a minimal medium and does not contain iron as it can reduce hexavalent chromium.

25% D-glucose solution

25ml of 25% glucose solution was prepared by mixing 6.25g glucose in 25ml distilled water and filter sterilized.

Preparation of VB Broth

VB Broth was prepared by aseptically mixing 4ml of VB concentrate, 4 ml of 25% glucose solution and 192 ml of distilled water.

Preparation of VB Agar

VB agar was prepared by adding 1.5g of agar to 100ml of VB broth.

Preparation of various concentrations of Chromium

Chromium in the form of potassium dichromate was prepared in concentrations of 100µg/ml, 200µg/ml, 300µg/ml and 400µg/ml.

Chromium utilization

The organisms were allowed to grow aerobically in VB broth having different concentrations of chromium (100, 200, 300, 400 µg/ml) at 37°C.

Table 1. Biochemical tests

S.No	Biochemical Tests	*Micrococcus sp.*	*S. marscens*	*Staphylococcus* sp.
1.	Catalase	+	+	+
2.	Oxidase	+	+	-
3.	Indole production	-	-	-
4.	Methyl Red	+	-	+
5.	Voges Proskauer	-	+	+
6.	Citrate utilization	+	+	-
7.	Glucose fermentation	+	+	+
8.	Nitrate reduction	+	+	+
9.	Coagulase	-	-	+

Table 2. Decolouration of broth

S.No	Organism	Concentration (µg/ml)			
		100	200	300	400
1.	*Micrococcus sp.*	GW	GW	GPY	GPY
2.	*S. marscens*	GW	GPY	GPY	GY
3.	*Staphylococcus sp.*	GW	GW	GW	GPY
4.	*Microsporum sp.*	GW	GPY	GPY	GY

GY- Growth, colour of medium remained yellow
GPY- Growth, medium colour turned to pale yellow
GW- growth, medium colour turned white

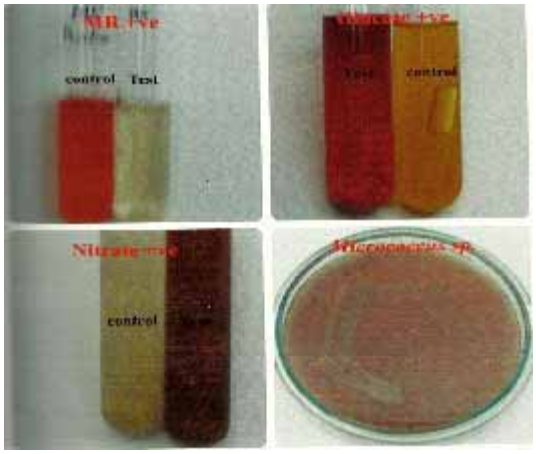

Fig. 1. Confirmatory test for *Micrococcus sp.*

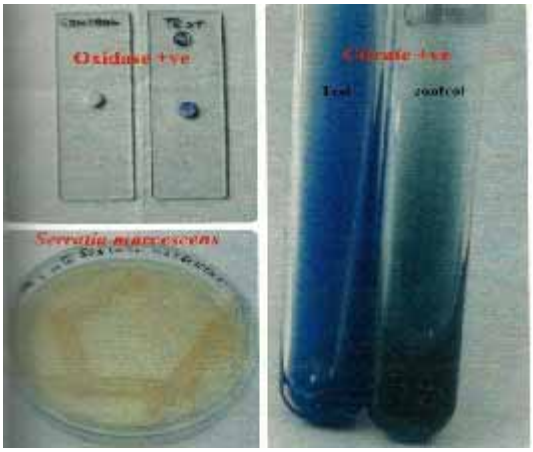

Fig 2. Confirmatory test for *Serratia marcescens*

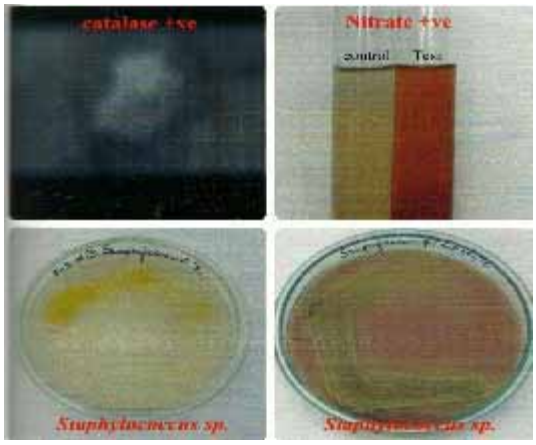

Fig. 3. Confirmatory test for *Staphylococcus sp.*

Fig. 4. *Microsporum sp.*

Chromium estimation by DPC method

The diphenyl carbazide(DPC) method is a sensitive and accurate method which relies on the redox reaction between chromate ions and DPC. Hexavalent chromium reacts with DPC under acid conditions to form red-violet colour. In acidic condition, chromate ions oxidize DPC to diphenyl carbazone to produce a red-violet complex. Results were quantified colorimetrically or by visual comparison with standards which are calibrated in ppm(mg/l) Cr_2O_4.

2ml of the broth was taken to which 40µl of DPC solution was added and mixed. Sulphuric acid(10%) was added to give a pH of 2+/- 0.5. The solution was diluted to 3ml with double distilled water and let stand for 5 to 10 mins for full color development. Absorbance was measured at 540nm.

Comparative study

The chromium reduction ability of the *Staphylococcus sp*. isolate was compared with a lab-strain of standard *Staphylococcus sp*. and the results were tabulated.

Effect of chromate concentration on pigment production

The effect of chromate on pigment production was studied in *Staphylococcus sp*., and *Serratia marcescens*.

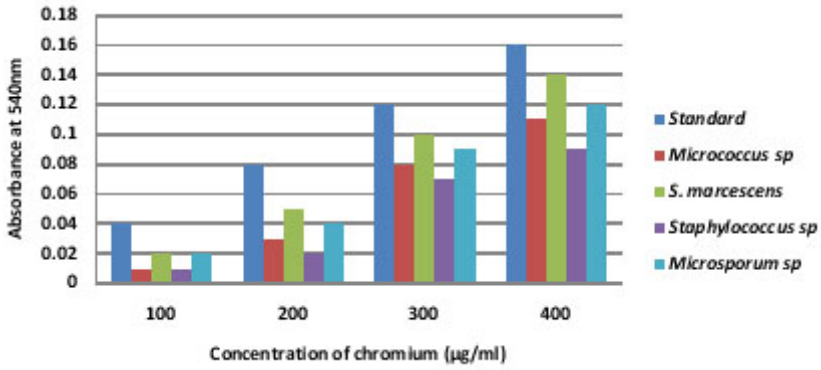

Fig. 5. Estimation of chromium reduction by different microorganisms (DPC method)

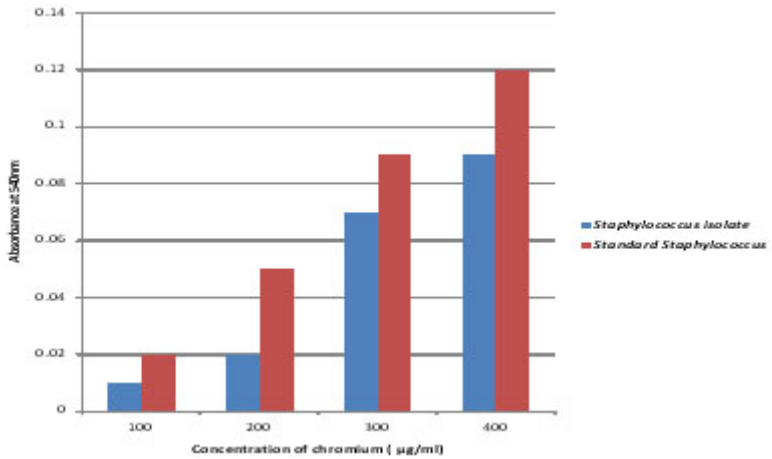

Fig 6. Comparison of chromium reduction ability of isolated *Staphylococcus sp* and standard *Staphylococcus sp* (DPC method)

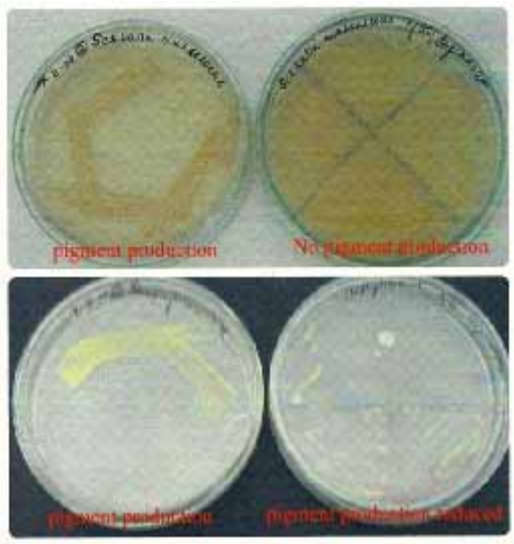

Fig. 7. Effect of chromium concentration on pigment production

RESULTS AND DISCUSSION

Three types of bacterial colonies were isolated and identified through simple staining and Gram staining as *Micrococcus*- Cocci, Gram positive (Fig 1), *Serratia marcescens*- Rods, Gram negative (Fig 2) , *Staphylococcus*- Cocci, Gram positive (Fig 3). They were subjected to further biochemical tests and confirmed as in Table 1. The fungal isolate was identified via colony morphology (colour, size and shape) and microscopic examination (lactophenol cotton blue staining) to be *Microsporum sp* (Fig. 4). After 15 days of incubation at 37°C, the colour change was observed as tabulated in Table 2. As can be seen from Fig 5., all the 4 isolates were able to detoxify chromium. Among these isolates, *Staphylococcus sp* had the strongest ability to detoxify high concentrations of chromium. Therefore, this activity of *Staphylococcus sp* was compared with lab-strain *Staphylococcus sp* and as can be seen from Fig 6., the isolate from tannery effluent had more pronounced activity in detoxifying chromium. The pigment production of *Serratia marcescens* and *Staphylococcus sp.* was affected (reduced) upon exposure to high concentrations of chromium as seen in Fig 7.

CONCLUSION

Heavy metal pollution of ground and surface waters by industrial effluents has become a serious threat to the environment especially in developing countries. One example of heavy metal pollution is the high chromium containing liquid effluents discharged from tanneries. Though many conventional chemical methods are currently being practiced, biotechnological methods are becoming attractive alternatives, as they are economical and eco-friendly. Only few microorganisms are able to withstand the toxic conditions of tannery effluent. The four isolates from the effluent were among those few organisms which possess the ability to grow under toxic conditions and also to detoxify chromium, one of the hazardous metals used in tanning process. This paves way for in situ bioremediation as the best method for treating tannery effluents with high chromium content.

REFERENCES

1. Sumit Yadav, O. P. Shukla & U. N. Rai. Chromium Pollution and Bioremediation. *Enviro News*, Archives, 2005; **11**(1).
2. Indu Shekhar Thakur. Environmental Biotechnology: Basic Concepts and Applications. I. K. International Pvt Ltd, 2006; 403.
3. Adel M. Zayed and Norman Terry. Chromium in the environment: factors affecting biological remediation. *Plant and Soil*. 2003; **249:** 139–156.
4. Shen, H., and Y.-T. Wang**.** Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. *Appl. Environ. Microbiol*., 1993; **59:** 3771–3777.
5. Camargo FA, Bento FM, Okeke BC, Frankenberger WT, 2003. Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. *J Environ Qual*., 2003; **32(4)**: 1228-33.
6. Mondaca MA, Campos V, Moaraga R, Zaror CA. Chromate reduction in *Serratia marcescens* isolated from tannery effluent and potential application for bioremediation of chromate pollution. *Sc. World J*., 2002; **2**: 972-977.
7. Pattanapipitpaisal P, N. Brown and L. Macaskie, Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site, *Applied Microbiology and Biotechnology* , 2001; **57(**1-2**):** 257-261
8. Wang, Tsukasa Mori,T Kiyoshi Toda, and Hisao Ohtake, Membrane-Associated Chromate Reductase Activity from *Enterobacter cloacae. Journal of Bacteriology*, 1990; **172**(3): 1670-1672.
9. Guha H, Jayachandran K, Maurrasse F, 2001. *Kinetics of chromium* (VI) reduction by a type strain *Shewanella alga* under different growth conditions. *Environ Pollut*., 2001; **115**(2): 209-18.
10. Campos, J., Martinez-Pacheco,M., and Cervantes, C.,1995.Hexavalent-chromium reduction by a chromate-resistant *Bacillus sp*.strain. *Antonie Van Leeuwenhoek.* 1995; **68**: 203-208.