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Chromium Resistant Yeast with Multi-Metal Resistance Isolated from Industrial Effluents and their Possible Use in Microbial Consortium for Bioremediation of Wastewater*

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Abstract.- Chromium (Cr) resistant yeasts with minimum inhibitory concentration of Cr^{6+} of 2.0 mg/mL were isolated from industrial effluents of tanneries, which could also tolerate 1.0-1.3 mg/mL of Pb^{2+}, 0.6-0.8 mg/mL of Cd^{2+} and Cu^{2+}, 0.4-0.5 mg/mL of Co^{2+}, 0.3-0.6 mg/mL of Ni^{2+}, 0.2 mg/mL of Hg^{2+} and 0.3-0.5 mg/mL of Zn^{2+}. The isolates showed optimum growth at 30°C and pH 5.5-6.0. All yeast isolates showed typical growth pattern except for the lag phase which got extended in the presence of Cr. The yeast isolates could remove 65-80% of Cr^{6+} from the medium after 72 hours of incubation. The yeast isolates absorbed 25-35% of Cr^{6+}, whereas 30- 40% of the Cr^{6+} was adsorbed on the cell surface. The yeast isolates can be exploited for bioremediation of chromium containing wastes, since they seem to have the potential to accumulate the toxic metal from the environment.

Key words: Bioremediation, chromium, heavy metal resistance, yeast, chromium up-take.

INTRODUCTION

All metals at elevated concentrations are potentially toxic, whether or not they are biologically essential at more moderate levels (Gadd, 1992). Metal toxicity can be readily observed as inhibition of growth or metabolic activity in metal-treated micro-organisms, and metal exposure of higher organisms is associated with a range of harmful effects. Chromium (Cr) is a highly toxic non-essential metal which is used on a large scale in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, chromium chemicals production and pulp and paper production. Often wastes from such industries are used as fill material (Salunkhe *et al.*, 1998). At many such sites, leaching and seepage of Cr^{6+} from the soil into the ground water poses a considerable health hazard. Cr exposure has been linked to genotoxicity, carcinogenicity and allergenicity (Dayan and Paine, 2001).

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Cr is a metal contaminant that, in nature, exists primarily as the soluble, highly toxic Cr^{6+} anion and the less soluble less toxic Cr^{3+}, the trivalent cation. Reduction/oxidation reactions between the two states are thermodynamically possible under physiological conditions (Arias and Tebo, 2003), thus chromate and Cr^{3+} are both biologically important ions. Chromate is more toxic than Cr^{3+}, so beneficial functions of Cr can only be performed by Cr^{3+} (Nielsen, 1998).

Moreover, Cr toxicity towards yeasts and other micro-organisms is of interest in its own right, from both environmental and biotechnological perspectives (White *et al.*, 1998; Cervantes *et al.*, 2001). This laboratory has embarked upon isolating and assessing the potential ability of various microorganisms to remove heavy metals from the industrial effluents (Shakoori *et al.*, 1999, 2000; Haq *et al.*, 2000, 2001; Rehman and Shakoori, 2001; Rehman *et al.*, 2007). As a useful means for bioremediation of environmental Cr contamination, yeasts were used to treat Cr-containing effluents in order to remove toxic compounds from waters and soil (Haq and Shakoori, 1998; Batic and Raspor,

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1998; Haq *et al.*, 2001).

Microbial reduction of toxic hexavalent chromium has practical importance, because biological strategies provide green technology that is cost-effective (Ganguli and Tripathi, 2002). Cr uptake and bioremediation by yeast are gaining much attention since these eukaryotic microorganisms have proven to be useful in biotechnological practice. Yeast has been applied in the management of Cr-containing waste as well as in nutritional supplementation of this trace metal (Barnhart, 1997). In this paper, we report the of Cr^{6+} resistant yeast from Kasur and their ability to remove Cr from the environment.

MATERIALS AND METHODS

Sample collection

Water samples of the industrial effluents from ponds getting wastes of tanneries in Kasur city, about 54 Km south-east of Lahore were collected in sterilized screw caped glass bottles. Some physical parameters of wastewater *viz*., pH and temperature were measured (APH, 1992).

Isolation of Cr resistant yeast

The streaking technique described by Benson (1994) was used to obtain pure culture of heavy metal resistant yeast. A series of dilutions were prepared from the industrial effluent *i.e.,* 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000. From each dilution, 20 µl were spread on five different YEPD agar plates containing 50 µg/mL of Cr^{6+}. The plates were incubated at 30°C for 48 hours. The colonies different in their shape, color, size and surface characteristics were picked and streaked on separate agar plates containing 100 µg/mL of chromium. This procedure was repeated with higher concentration of chromium in the medium. Every time, 100 µg metal ions were added more than that of the previous step, until the minimum inhibitory concentration (MIC) of Cr was obtained for each isolate. Aqueous solution of potassium dichromate was used as a source of Cr^{6+}.

Morphological and biochemical characteristics of the yeast isolates

For morphological and biochemical characterization the yeast isolates were tested for colony and cell morphology, Gram staining, formation of mycelium and sporulation, carbon assimilation, acid production from different sugars, starch production, nitrate reduction, urease activity, ester production, growth on 50% glucose medium, 5% glucose and 10% NaCl containing media, and Diazonium blue B (DBB) test for which procedures of Benson (1994) were followed.

Determination of optimum growth conditions

For determination of optimum temperature, 5 mL YEPD broth was added in five sets, each of three test tubes, autoclaved, inoculated with 100 µL of log phase growing cells and incubated at different temperatures *i.e.,* 20ºC, 25ºC, 30ºC, 35ºC and 40ºC. After 12 hours, growth of yeast was assessed by measuring absorbance at 600 nm. A graph was plotted between O.D. and temperature.

For ascertaining optimum pH, 5 mL YEPD broth was added in 3 sets, each of thirteen test tubes and with different pH *viz*. 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, autoclaved, inoculated with 100 µL of log phase growing yeast cell and incubated at 30ºC for 16 hours. Growth was assessed by measuring absorbance at 600 nm. A graph was plotted between O.D. and pH.

YEPD broth medium was used for the determination of optimum inoculum size. In three sets, each of four test tubes, 6 ml of the medium was poured and autoclaved. The medium was inoculated with log phase growing yeast culture at a concentration of 5% (300 µL), 10% (600 µL), 20% (1200 µL) and 40% (2400 µL) and placed in shaking water bath at 30ºC. After 16 hours, O.D. was taken at 600 nm. A graph was plotted between O.D. and inoculum size.

Determination of growth curve

Growth curves of yeast isolates were determined with and without heavy metals. YEPD broth (100 mL) was taken in five 250 mL flasks and sterilized. One flask was the control, whereas the other five had metals Cr^{6+}, Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+} at a concentration of 100 µg/mL. The medium was inoculated with the 10% inoculum of log phase yeast culture. The flasks were incubated in shaking

incubator at 30°C. The aliquots (2mL) of incubated medium taken out at regular interval of four hour for 24 hours and the extent of yeast growth was determined by taking optical density (O.D) at 600 nm.

Determination of cross heavy metal resistance

The heavy metal resistant yeast isolates were checked for their ability to resist other heavy metals. YEPD medium was prepared with different concentrations of cadmium chloride to get 0.1-1.5 mg/mL of Cd^{2+} concentration, copper chloride to get 0.1-3.5 mg/mL of Cu^{2+} concentration, potassium dichromate to get 0.1-3.0 mg/mL of Cr^{6+} concentration, lead acetate to get 0.1-4 mg/mL of Pb^{2+} concentration, mercuric chloride to get 0.1-0.7 mg/mL of Hg^{2+} concentration, cobalt chloride to get 0.1-0.9 mg/mL of Co^{2+} concentration, nickel acetate to get 0.1-1.4 mg/mL of Ni^{2+} concentration, and zinc sulphate to get 0.1-1.2 mg/mL of Zn^{2+} concentration. The YEPD media and metal ion solutions were prepared and autoclaved in separate flasks. The each metal solution was mixed thoroughly in its respective medium and poured into sterilized Petri plates. After inoculation, the plates were incubated at 30°C for a maximum period of 15 days.

Metal processing ability of yeast isolates

For determining the metal processing ability, the yeast isolates were grown in YEPD medium containing 100 μg/mL of Cr^{6+} at optimum pH and temperature in culture flasks. A control was also run having 100 μg/mL of metal concentration but without yeast inoculation. The samples to be estimated were taken out of the flask at regular interval of every four hours until 72 hours of incubation, and centrifuged at 3000 rpm for 15 minutes to spin down the cells. The absorbance of the supernatant was taken with the help of AA1275 atomic absorption spectrophotometer at wave length 357.9 nm and the concentration of metal in the supernatant was estimated. A graph was plotted between the time interval and chromium content.

Adsorption and absorption of heavy metals

In order to ascertain, whether the processing involves adsorption or absorption, the following methodology was adopted.

Adsorption of chromium

Yeast cells were grown in YEPD medium containing 100 μg/mL of the metal. After regular intervals, *i.e*. every 4 hours until 24 hours, the cells were harvested from 5 mL of culture by centrifugation at 4350 × g for 15 min and washed three times with isotonic solution (0.9% NaCl). The cells were resuspended in 10 mL of hypertonic solution (9% NaCl) and incubated at 30°C for overnight. Afterwards it was centrifuged at 3000 rpm (1090 × g) for 15 min. The supernatant was taken and the amount of metal was estimated by atomic absorption spectrophotometer.

Absorption of chromium

The cells in pellet obtained in the above experiment were lysed by treating with 5 mL mixture of concentrated H_2SO_4 and HNO_3 (1:1) and the amount of metals absorbed by yeast was measured by atomic absorption spectrophotometer. Experiment was performed in triplicate.

Statistical analysis

All observations and estimations were done in triplicate. At least three flasks were usually maintained for each metal. The average of control and experimental groups were compared and significant differences evaluated by using student's "t" test of significance (Sokal and Rohlf, 1981).

RESULTS

Heavy metal resistant yeast strains

Five water samples were collected, from the wastewater of Kasur tanneries. Eight Cr-resistant yeast isolates (CMBLY-Cr1 - CMBLY-Cr8) were isolated for further studies. The wastewater samples had pH 8.6 and temperature of 30°C.

The MICs of Cr^{6+} resistant yeast strains was checked, and yeast isolates CMBLY-Cr1, Cr4 and Cr8 showed maximum tolerance at a concentration of 2.0 mg/mL of Cr^{6+}.

Identification of yeast isolates

Tables I and II show the morphological and biochemical characters of yeast isolates after 48 hours of incubation. On the basis of morphological

and biochemical characteristics CMBLY-Cr1, was identified as *Candida etchellsii*, CMBLY-Cr4 as *Rhodotorula graminis* and CMBLY-Cr8 as *Candida glabrata.*

Table I.- General colony and cell morphology of different heavy metal resistant yeast isolates (CMBLY Cr1, 4, 8) from wastewater of Kasur Tanneries.

Yeats strain	Cr1	Cr4	Cr8
Colony color	Creamy white	Pink	Creamy white
Colony shape	Smooth, oval entire	Oval, convex, mucous, smooth	Glossy, smooth
Cell form	Single	Single	Chams
Cell size (µm)	2.6-5.7	4.0-5.7	2.3-5.6
Cell shape	Oval	Oval	Oval
Buds	Bipolar	Multi	Elongated
Ascospore formation	-	-	-
Pseudomycelium	-	-	-
Identification	*Candida etchellsii*	*Rhodotorula graminis*	*Candida glabrata*

Cross resistance of multi heavy metals

Cross resistance of Cr^{6+} resistant yeast isolates were determined against other heavy metals *i.e.*, Co^{2+}, Cr^{6+}, Hg^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (Table III). Chromium resistant yeast isolates showed maximum resistance against Cr^{6+} (2.0 mg/mL) and minimum resistance against Hg^{2+} (0.2 mg/mL). They could tolerate 1.0- 1.3 mg/mL of Pb^{2+}, 0.6-0.8 mg/mL of Cd^{2+} and Cu^{2+}, 0.4-0.5 mg/mL of Co^{2+}, 0.3-0.6 mg/mL of Ni^{2+} and 0.3-0.5 mg/mL of Zn^{2+}.

Optimum growth conditions

The growth conditions of heavy metal resistant yeast isolates were optimized. The most suitable temperature for chromium resistant yeast isolates was found to be 30°C. The maximum growth of CMBLY-Cr1 was found at pH 6.0, while CMBLY-Cr4 and CMBLY-Cr8 showed their maximum growth at pH 5.5. The amount of yeast inoculum introduced into the medium had significant effect on the growth pattern of different yeast isolates. The 20% inoculum size of the total yeast culture was most suitable for the optimum growth of CMBLY-Cr1, while it was 10% for CMBLY-Cr4 and CMBLY-Cr8.

Growth curves

The growth curves with and without Cr^{6+} (100 µg/mL) treatment were determined. Figure 1 clearly shows the characteristic phases during the growth of culture. The control growth curves showed lag phase of 4-6 hours, and log of 20-36 hours, whereas in yeast cultures under Cr^{6+} stress

Table II.- Biochemical characterization for the identification of metal resistant yeast isolates (CMBLY Cr1, 4, 8).

Biochemical tests	Yeast isolates		
	Cr1	Cr4	Cr8
Fermentation test:			
Glucose	+	-	+
Glactose	+	-	+
Starch	-	-	-
Maltose	+	-	+
Sucrose	-	-	-
Lactose	-	-	-
Raffinose	-	-	-
Carbon Assimilation test:			
Glucose	+	+	+
Glactose	-	+	-
Fructose	+	+	+
Sucrose	-	+	-
Citric acid	+	+	+
Maltose	-	+	-
Lactose	-	-	-
Raffinose	-	-	-
Soluble starch	-	-	-
Growth at			
20°C	+	+	+
25°C	+	+	+
30°C	+	+	+
35°C	+	-	+
40°C	-	-	-
Ester production test	-	-	-
Starch production test	-	-	-
Nitrate reduction test	N	+	N

Growth on 50% glucose medium	+	+	+
Growth on 5% and 10% glucose	+	+	+
Growth on 60% glucose	+	-	+
Urea hydrolysis	-	+	-
Diazonium blue B	-	+	-
Citrate utilization test	+	-	+

+, positive test;-, negative test; N, not done.

Table III.- Cross heavy metal resistance shown as MIC (mg/mL) of chromium resistant yeast isolates from industrial wastewater against other heavy metals.

	CMBLY Cr1	CMBLY Cr4	CMBLY Cr8
Chromium	2.0	2.0	2.0
Cadmium	0.8	0.6	0.8
Cobalt	0.5	0.5	0.4
Copper	0.6	0.6	0.8
Mercury	0.2	0.2	0.2
Lead	1.2	1.2	0.3
Nickel	0.4	0.4	0.3
Zinc	0.4	0.3	0.4

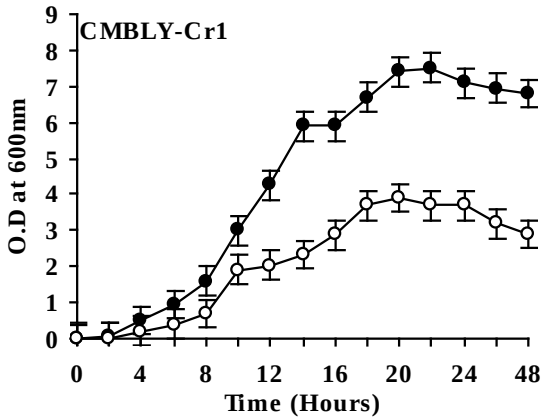
showed 6-8 hours of lag phase and 18-22 hours of log phase. After this for few hours the O.D of the culture and the number of yeast cells remained constant, which was the stationery phase. After this the growth was declined rapidly.

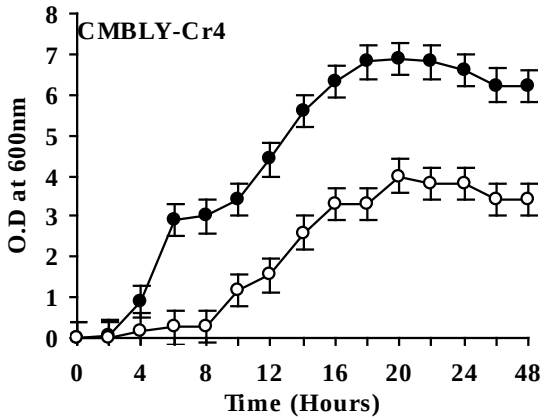
Processing of metals by yeast isolates

Figure 2 shows the ability of different Cr^{6+} resistant yeast isolates to reduce the level of Cr^{6+} in the YEPD medium. Chromium resistant isolates removed 65-80% of Cr^{6+} from the medium after 72 hours of incubation. CMBLY-Cr4, and Cr8 showed maximum removal of Cr^{6+}. They removed 70-80% of Cr^{6+} from the medium after 72 hours of incubation.

Adsorption and absorption of heavy metals

The metal ions can adsorb onto the cell surface or can accumulate inside the cells. Table IV shows the percentage adsorption and absorption of chromium in chromium resistant yeast strains after 24 hours of incubation. The percentage absorption of Cr^{6+} in chromium resistant yeast isolates was 25-35%. CMBLY- Cr4 and Cr8 absorbed the maximum amount *i.e.* 35% of Cr^{6+}. It was observed that chromium resistant yeast cells could adsorb 30-40% of the Cr^{6+} after 24 hours. CMBLY- Cr4 and Cr8 adsorbed the highest amount of Cr^{6+} *i.e.* 40% after 24 hours of incubation.





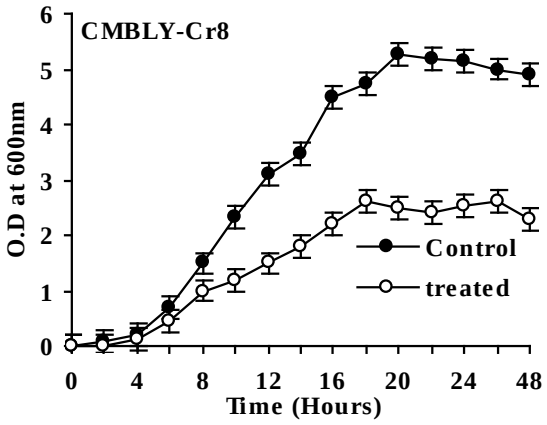
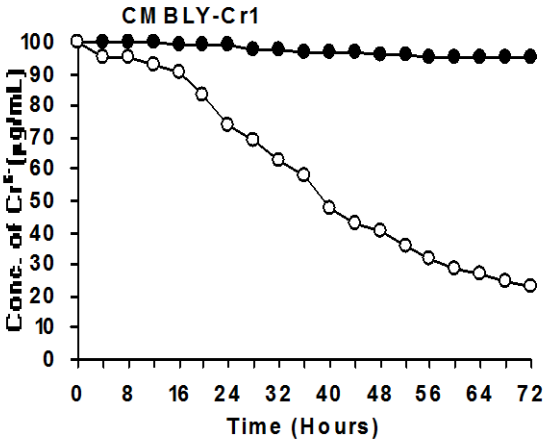
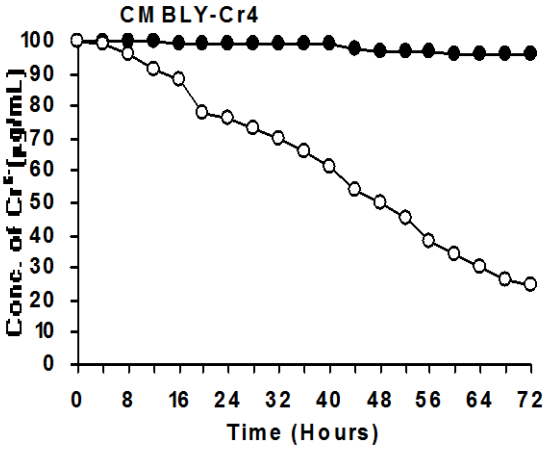


Fig. 1. Effect of Cr^{6+} on the growth of Cr^{6+} resistant yeast, isolated from the wastewater of tanneries, depicted by the number of cells/mL, after 48 hours of incubation at 30°C in YEPD medium with and without 100 µg/mL of Cr^{6+}.





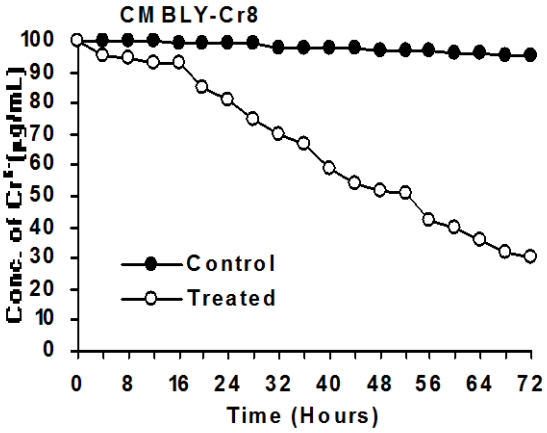


Fig. 2. Chromium processing capability of metal resistant yeast isolates from industrial wastewater. The isolates were grown for 72 hours in YEPD medium containing 100 µg/mL of chromium (treated). The control culture medium contained chromium but no organism.

DISCUSSION

Microorganisms have evolved various measures to respond to heavy-metal stress via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation–reduction reactions (Rai *et al.*, 1981a,b; Macaskie, 1990; Huang *et al.*, 1990; Avery and Tobin, 1993; Brady and Duncan, 1994; Brady *et al.*, 1994; Krauter *et al.*, 1996; Veglio *et al.*, 1997). They have proven capability to take up heavy metals from aqueous solutions, especially when the metal concentrations in the effluent range from less than 1 to about 20 mg/L (Brierley, 1990).

In the present study, heavy metal resistant yeasts were isolated from the tannery effluents of Kasur area. Their presence in the industrial effluents prompted this study for heavy metal tolerance and their potential for bioremediation of wastewater. Hexavalent chromium (Cr^{6+}) resistant yeast strains *Candida* sp. from tannery wastes, *Rhodosporidium* sp. from metallurgical industry's wastes (Baldi *et al.*, 1990) and *Rhodosporidium toruloids* (Baldi and Pepi, 1991) from industrial waste have been reported.

Microorganisms can tolerate high

concentration of metals (Nies, 2003; Angle *et al.*, 1993). At doses higher than 2.0 mg/mL Cr^{6+}, no growth was observed for any of the isolated chromium resistant yeast strains in this study, which indicated that this concentration blocked cell division. It is pertinent here to note that a number of yeast strains have been reported with Cr^{6+} resistance. For example, *Candida* sp., isolated from tannery wastes has been found having MIC of 0.5 mM (25 μg/mL) Cr^{6+}, while for *Rhodosporidium* sp. from metallurgical wastes, the MIC was 8mM (400 μg/mL) Cr^{6+}, while both strains were grown in the Sabouraud Broth medium (Baldi and Pepi, 1991). These MIC values were much less as compared to those found for the yeasts in the present investigation. Apart from species differences, one factor is the nature of medium. Medium used in this study did not contain sulfur, whereas Sabouraud medium containing SO_4^{2-} and demonstrate that the sulfate enhance sulfur transport system and also Cr^{6+}

Table IV.- Percentage adsorption and absorption of chromium in yeast isolates.

Yeast isolates	% adsorption and absorption of chromium by yeast isolates after 24 hours of incubation at 30°C													
	0 hr		4 hr		8 hr		12 hr		16 hr		20 hr		24 hr	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
Y-Cr1	0.0	0.0	9.1	7.2	16.9	16	22.8	27.2	30.4	29.1	33.3	31.1	35.1	33.5
Y-Cr4	0.0	0.0	7.9	2.4	10	16	15.2	19.2	19.2	20.2	21.2	20.2	30.4	25.1
Y-Cr8	0.0	0.0	11	7.5	16.3	17.3	20.6	20.6	25.2	27	29.1	29.6	34.7	34.6

Ab, Absorption; Ad, Adsorption.

uptake rendering the species more sensitive to the level of Cr^{6+} in the medium. Baldi *et al.* (1990) reported chromate resistance in *Candida*, which could grow in glucose peptone broth medium containing upto 10 mM (500 μg/mL) of Cr^{6+}. *Pichia guilliermondii* was able to grow in the presence of 0.5 mM Cr^{6+} (Ksheminska *et al.*, 2003).

By optimizing the growth conditions the number and efficiency of the microorganisms can be increased for their use in bioremediation of polluted environment (Haq and Shakoori, 2000). For all the yeast isolates, maximum growth was observed at 30°C, which is the optimum temperature for yeast (Amanchukwu *et al.*, 1989). The binding of metals depends on temperature (Blackwell *et al.*, 1995). Several factors, including organic matter, pH, temperature and redox potential might influence the solubility of heavy metals (Sauve *et al.*, 1997). Bosecker (1993) found that, among various filamentous fungi, *Penicillium funiculosum* was able to extract more than 50% Ni, and 75% Zn from test solutions containing 100 mg/L of a metal at pH 6.6 and 6.5, respectively. The pH dependent inhibition of 22 metals has been investigated for the yeast *Saccharomyces cerevisiae* by Pearce and Sherman (1999). Batic and Raspor (1998) have reported that chromium biosorption on the cell surface points to a correlation between the pH medium and the chromium accumulation on the cell surface.

In the present study yeast isolates were able to remove 80% of Cr^{6+} after 72 hours of incubation when they were cultured in the presence of 100 μg/mL of Cr^{6+}. Rate of the metal clearing in the medium was highest at 48 hours sampling period in all the isolates. Higher concentrations of the metals are likely to exert toxic effects on the microbes. Bioremediation of the metal containing effluents by these isolates seems possible but for dilute effluents only. Pepi and Baldi (1992) have reported certain Cr^{6+} resistant and Cr^{6+} -sensitive yeast strains, which did not reduce Cr^{6+} to Cr^{3+} but have some efficiency to accumulate it. Whether the yeast isolates studied in this investigation, possess reduction abilities or they simply uptake the metal from the medium and accumulate with resultant lowering of the metal concentration in the medium has to be determined.

REFERENCES

AMANCHUKWU, S.C., OBAFEMI, A. AND OKPOKAWASILI, G.C., 1989. Hydrocarbon degradation and utilization by a palmwine yeast isolate. *FEMS Microbiol. Lett.*, **57:** 151-154.

ANGLE, J.S., CHANEY, R.L. AND RHEE, D., 1993. Bacterial resistance to heavy metals related to extractable and total metal concentration is soil and media. *Soil Biol. Biochem.,* **25:** 1443-1446.

APH, 1992. *Standard methods for the examination of water and wastewater*, 18th ed. APHA, Washington, DC.

ARIAS, Y.M. AND TEBO, B.M., 2003. Chromium reduction by sulfidogenic and non sulfidogenic microbial consortia. *Appl. environ. Microbiol.,* **69:** 1847-1853.

AVERY, S.V., 2001. Metal toxicity in yeasts and the role of oxidative stress. *Adv. appl. Microbiol.,* **49:** 111-142.

AVERY, S.V. AND TOBIN, J.M., 1993. Mechanism of adsorption of hard and soft metal ions to a *Saccharomyces cerevisiae* and influence of hard and soft anions. *Appl. environ. Microbiol.,* **59: 2**851-2856.

BALDI, F. AND PEPI, M., 1991. Study of metal and non-metal resistances in chromate-resistant yeasts isolated from industrial wastes. In: *Proc. Int. Symp. Environm. Biotechnol* (eds. H. Verachtert and W. Verstraete), vol. 1, pp. 255-258. Ostend.

BALDI, F., VAUGHAN, A.M. AND OLSON, G.J., 1990. Chromium (VI) resistant yeast isolated from a sewage treatment plant receiving tannery wastes. *Appl. environ. Microbiol.,* **56:** 913-918.

BARNHART, J., 1997. Occurrences, uses, and properties of chromium. *Regul. Toxicol. Pharmacol.,* **26:** 3–7.

BATIC, M. AND RASPOR, P., 1998. Uptake and bioaccumulation of chromium (III) in yeast. *Fd. Technol. Biotechnol.,* **4:** 291–297.

BATIC, M. AND RASPOR, P., 2000. Effect of cultivation mode on a bioprocess for chromium yeast biomass enrichment, *Pflugers Arch. Eur. J. Physiol.,* **439:** R73–R75.

BENSON, H.J., 1994. *Microbiological applications, complete version: Laboratory manual in general microbiology*. Wan C. Brown Publishers. Dubuque Melbourn, Oxford.

BLACKWELL, K.J., SINGLETON, I. AND TOBIN, J.M., 1995. Metal cation uptake by yeast. *Appl. Microbiol. Biotechnol.,* **43:** 579-584.

BOSECKER, K., 1993. Bioleaching of silicate manganese ores. *Geomicrobiol. J.,* **11:** 195-203.

BRADY, D. AND DUNCAN, J.R., 1994. Bioaccumulation of metal cations by *Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol.,* **41:** 149-154.

BRADY, D., GLAUM, D. AND DUNCAN, J.R., 1994. Copper tolerance in *Saccharomyces cerevisiae. Lett. Appl. Microbiol. Biotechnol.,* **18:** 245-250.

BRIERLEY, C.L., 1990. Bioremediation of metal-contaminated surface and groundwaters. *Geomicrobiol. J.,* **8:** 201-205.

CERVANTES, C., CAMPOS-GARCIA, J., DEVARS, S., GUTIERREZ-CORONA, F., LOZA-TAVERA, H., TORRES-GUZMAN, J.C. AND MORENO-SANCHEZ, R., 2001. Interactions of chromium with microorganisms and plants. *FEMS Microbiol. Rev.,* **25:** 335-347.

DAVIS, C.M. AND VINCENT, J.B., 1997a. Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry,* **36:** 4382-4385.

DAVIS, C.M. AND VINCENT, J.B., 1997b. Isolation and characterization of a biologically active chromium oligopeptide from bovine liver. *Arch. Biochem. Biophys.,* **339:** 335-343.

DAYAN, A.D. AND PAINE, A.J., 2001. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. *Human Exp. Toxicol.,* **20:** 439–451.

GADD, G.M., 1992. Metals and microorganisms – a problem of definition. *FEMS Microbiol. Lett.,* **100:** 197–203.

GANGULI, A. AND TRIPATHI, A.K., 2002. Bioremediation of toxic chromium from electroplating effluent by chromate reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. *Appl. Microbiol. Biotechnol.,* **58:** 416–420.

HALLIWELL, B. AND GUTTERIDGE, J.M.C., 1999. Free radicals. In: *Biology and medicine*, 3rd edn. Oxford University Press, Oxford:

HAQ, R.U. AND SHAKOORI. A.R., 1998. Microbiological treatment of industrial wastes containing toxic chromium involving successive use of bacteria, yeast and algae. *World J. Microbiol. Biotechnol.,* **114:** 583–585.

HAQ, R.U. AND SHAKOORI, A.R., 2000. Microorganisms resistance to heavy metals and toxic chemicals as indicators of environmental pollution and their use in bioremediation. *Folia Biol.,* **48:** 143-147.

HAQ, R.U., AFSHAN, A., SHAKOORI, F.R. AND SHAKOORI, A.R., 2001. Dichromate resistant yeast isolated from industrial wastewater and their role in bioremediation of heavy metals. *Proc. Pakistan Congr. Zool*., **21**: 181-226.

HUANG, C.P., HUANG, C.P. AND MOREHART, A.L., 1990. The removal of Cu (II) from dilute aqueous solutions by *Saccharomyces cerevisiae. Water Res*., **24:** 433-439.

KRAUTER, P., MARTINELLI, R., WILLIAMS, K. AND MARTINS, S., 1996. Removal of Cr (VI) from ground water by *Saccharomyces cerevisiae. Biodegradation,* **7:** 277-286.

KSHEMINSKA, H., JAGLARZ, A., FEDEROVYCH, D., BABYAK, L., YANOVYCH, D., KASZYCKI, P. AND KOLOCZEK, H., 2003. Bioremediation of chromium by the yeast *Pichia guilliermondii*: toxicity and accumulation of Cr (III) and Cr (VI) and the influence of riboflavin on Cr tolerance. *Microbiol. Res.,* **158:** 59-63.

MACASKIE, L.E., 1990. An immobilized cell bioprocess for removal of heavy metals from aqueous flows. *J. Chem. Technol. Biotech.,* **49:** 330-334.

NATIONAL RESEARCH COUNCIL, 1989. *Recommended*

dietary allowances (10th ed.). National Academy Press, Washington, DC:

NIELSEN, F.H., 1998. Ultratrace elements in nutrition: Current knowledge and speculation. *J. Trace Elem. exp. Med.,* **11:** 251–274.

NIES, D.H., 2003. Efflux mediated heavy metal resistance in prokaryotes. *FEMS Microbiol. Rev.,* **27:** 313-339.

PAWLISZ, A.V., KENT, R.A., SCHNEIDER, U.A. AND JEFFERSON, C., 1997. Canadian water quality guidelines for chromium. *Environ. Toxicol. Water Qual.,* **12:** 123-183.

PEARCE, D.A. AND SHERMAN, F., 1999. Toxicity of copper, cobalt and nickel salts is dependent on histidine metabolism in yeast *Saccharomyces cerevisiae. J. Bact.,* **181:** 4774-4779.

PEPI, M. AND BALDI, F., 1992. Modulation of chromium (VI) toxicity by organic and inorganic sulfur species in yeast from industrial wastes. *BioMetals,* **5:** 179–185.

RAI, L. C., GAUR, J.P. AND KUMAR, H.D., 1981a. Phycology and heavy metal pollution. *Biol. Rev.,* **56:** 99-151.

RAI, L.C., GAUR, J.P. AND KUMAR, H.D., 1981b. Protective effects of certain environmental factors on the toxicity of zinc, mercury, and methylmercury to *Chlorella vulgaris. Environ. Res.,* **25:** 250-259.

REHMAN, A., SHAKOORI, F.R. AND SHAKOORI, A.R., 2007. Heavy metal resistant *Distigma proteus* (Euglenophyta) isolated from industrial effluents and its possible role in bioremediation of contaminated waste waters. *World J. Microbiol. Biotechnol.*, **29**: 753-758.

REHMAN, A. AND SHAKOORI, A.R., 2001. Heavy metal resistant *Chlorella* spp., isolated from tannery effluents and their role in remediation of hexavalent chromium in industrial waste water. *Bull. environ. Contam. Toxicol*., **66**: 542-547.

SALUNKHE, P.B., DHAKEPHALKAR, P.K. AND PAKNIKAR, K.M., 1998. Bioremediation of hexavalent chromium in soil microcosms. *Biotechnol. Lett.,* **20:** 749.

SAUVE, S., MCBRIDE, M.B. AND HENDERSHOT, W.H., 1997. Speciation of lead in contaminated soils. *Environ. Pollut.,* **98:** 149-155.

SHAKOORI, A.R., MAKHDOOM, M. AND HAQ, R.U., 2000. Hexavalent chromium reduction by a dichromate-resistant gram positive bacterium isolated from effluents of tanneries. *Appl. Microbiol. Biotechnol.*, **53**: 348-351.

SHAKOORI, A.R., REHMAN, A. AND HAQ, R.U., 2004. Multiple metal resistance in the ciliate protozoan *Vorticella microstoma*, isolated from effluents and its potential in bioremediation of toxic wastes. *Bull. environ. Contam. Toxicol*., **72**: 1046-1051.

SHAKOORI, A.R., TAHSEEN, S. AND HAQ, R.U., 1990. Chromium tolerant bacteria from industrial effluents and their use in detoxication of hexavalent chromium. *Folia Microbiol*., **44**: 50-54.

SOKAL, R.R. AND ROHLF, F.J., 1981. *Biometry. The principles and practice of statistics in biological research.* 2nd Ed. W.H. Freeman and Company, San Francisco, CA.

VEGLIO, F., BEOLCHINI, F. AND GASBARRO, A., 1997. Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacter* sp. *Proc. Biochem.,* **32:** 99-105.

WHITE, C. AND GADD, G.M., 1998. Accumulation and effects of cadmium on sulfate-reducing bacterial biofilms. *Microbiology,* **144:** 1407-1415.

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