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Chromium Tolerant Yeast Strains Isolated from Industrial Effluents and Their Possible Use in Environmental Clean-Up

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Environmental metal toxicity is a hazard of increasing significance because of the constant deposition of these pollutants in the biosphere as a result of heavy industrialization (Zhou et al. 1992). Heavy metal ions in small quantities are required for various physiological processes and normal functions of the cells in plants, animals and micro-organisms. Elevated levels of such metal ions are generally toxic and cause major damage to cell (Inouho et al. 1996). Experimental evidence indicate that Cr^{+6} is carcinogenic (Krishnamurthi and Nair 1991) and mutagenic (Nishioka 1975).

Many micro-organisms show adaptations to the otherwise toxic materials constantly released in their environment. A large variety of micro-organisms like bacteria, yeast, algae and fungi are found in waters receiving industrial effluents. They have developed strategies to resist, tolerate, metabolize and to detoxify these toxic substances (Parsek et al. 1995). Yeasts have previously been shown to acquire resistance to toxic metals (Brady et al. 1994, Olasupo et al. 1993, Gadd 1993, Mehra and Winge 1991). It is conceivable that the acquisition of tolerance to toxic metals by yeasts could be of use in metal bioaccumulation process (Brady and Duncan 1994).

Conventional methods for treatment of hexavalent chromium (Cr^{+6}) require either high energy or large quantities of chemicals and therefore more practical and cost effective methods are being explored (Ohtake and Hardoyo 1992). We have isolated a number of yeast strains from industrial effluents of tanning leather, steel and chemical industries, which not only resist high concentrations of chromium but also remove Cr^{+6} from the environment. These isolates can be used for the bioremediation of environment.

MATERIALS AND METHODS

Samples of industrial effluents were spread on the Petri plates containing YEPD

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medium (Oxoid, Hampshire, England; yeast extract l%, peptone 0.5%, dextrose 0.2%), and incubated at 30°C up to eight days. Antibiotics (Gentamicin, 0.1 μgmL^{-1}; Chloramphenicol, 1 μgmL^{-1}; Ampicillin, 1 μgmL^{-1}) were added to inhibit the growth of bacteria.

To check the Cr^{+6} resistance, the freshly prepared (10-14 hr incubation) liquid cultures of isolated yeasts were streaked on YEPD plates containing various concentrations of $K_2Cr_2O_7$, viz. 1.0, 1.5, 2.0, 2.5, and 3.0 $mgmL^{-1}$ and incubated at 30°C for visible growth.

For determination of optimum temperature, 5ml YEPD broth (pH 7.0) was inoculated with fresh yeast culture (14 hr incubation) and the growth was followed at 20, 25, 30, 35 and 40°C. For determination of optimum pH, the yeast isolate was grown in 5 mL YEPD medium with different pH such as 3, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0 and incubated in shaking water bath with 50 rpm at 30°C. Both experiments were run in triplicate and absorbance was checked after 10 hr of incubation by spectrophotometer at 600 nm.

To study the toxic effect of Cr^{+6} on the growth of yeast, 200 ml of YEPD liquid medium containing 100 μgmL^{-1} Cr^{+6} was inoculated with log phase growing culture (1.2×10^5 cells/inoculum) and was placed in shaking water bath at 50 rpm at 30°C. Samples were taken out every 2 hr up to 96 hr of incubation. Absorbance was recorded at 600 mn. YEPD medium without chromium was also inoculated with same amount of inoculum and was run in parallel as control. Cell size of both the normal and Cr-treated yeast was also measured with the help of ocular micrometer at 0, 12, 24 and 48 hr of incubation.

The resistance of Cr-resistant yeast isolates against other heavy metals was also checked. Petri plates with YEPD medium containing various concentrations of lead acetate, cadmium chloride, copper sulphate, mercuric chloride and cobalt chloride were streaked with isolated strains of yeast and incubated at 30°C for visible growth. The initial concentration used for lead acetate, cadmium chloride, copper sulphate, mercuric chloride and cobalt nitrite was respectively 0.5, 0.1, 0.05, 0.05 and 0.01 mg mL^{-1}

For ascertaining the ability of yeast isolates to process Cr^{+6}, yeast cells (14×10^4) were inoculated into 25 mL YEPD medium (pH 7.5) containing 100 μgmL^{-1} Cr^{+6} and incubated at 30°C. Samples were taken out after 48, 72 and 96 hr of incubation and processed for estimation of Cr^{+6} in the medium using di-phenyl carbazide method (Clesceri et al. 1989). Cr^{+6} containing media without yeast cell was run in parallel as control. The experiment was run in triplicate.

RESULTS AND DISCUSSION

Forty yeast strains were isolated from the samples of industrial wastes of leather, steel and tanning industries of Kasur (11 isolates), Kala Shah Kaku (8 isolates), Kot Lakh Pat (2 isolates), Sheikhupura Road (4 isolates), Ravi Road (1 isolate) and Karachi (14 isolates). These isolates showed variable growth response in the presence of Cr^{+6} in the medium. Some of the isolates (CMBLY 11, 12, 17, 23, 24, 32, 43, 45). were sensitive and could not grow at low doses of Cr (> 1.0 $mgmL^{-1}$). Others were resistant and could grow on 1.0 $mgmL^{-1}$ concentration of Cr. CMBLY 3, 10, 31, 50, 62, and 80 were highly resistant and could grow at 2.0 $mgmL^{-1}$ Cr (Table 1). Yeast resistance to toxic chromate has been reported previously from different laboratories (Baldi et al. 1990, Olasupo et al. 1993, Ono and Weng 1982). Some of the isolated strains were highly resistant and could grow at Cr concentration up to 3.0 $mgmL^{-1}$, which is much higher as compared with the results reported by other studies. Baldi (1990) reported yeast strains, which were resistant to 500 μgmL^{-1} Cr. In the study conducted by Ono and Weng (1982) many strains of *Saccharomyces cerevisiae* do not grow on YEPD agar containing 750 μgm^{-1} Cr_2O_3 but their mutants could grow up to 1000 μgmL^{-1} Cr_2O_3.

Table 1. Minimum inhibitory concentration of various yeast isolates to different heavy metals.

Yeast isolates	Lead (mg/mL)	Copper (μg/mL)	Mercury (μg/mL)	Cobalt (μg/mL)	Chromium (mg/mL)
CMBLY 3	3	200	200	200	2.0
CMBLY 10	2	100	200	200	2.0
CMBLY 30	2	100	50	200	1.5
CMBLY 31	2	200	100	300	2.0
CMBLY 33	2	200	100	300	1.0
CMBLY 36	3	-----	100	300	1.0
CMBLY 44	1	100	200	200	1.5
CMBLY 46	1	200	200	100	1.5
CMBLY 50	2	100	100	300	2.0
CMBLY 57	2	200	200	400	1.5
CMBLY 61	2	100	50	200	1.0
CMBLY 62	3	50	100	200	2.0
CMBLY 64	1.5	100	50	100	1.5
CMBLY 70	1.5	200	100	400	1.5
CMBLY 80	3	200	200	100	2.0

The colonies of yeast isolates appeared on YEPD agar medium at 30°C after incubation up to 48 hr. However, when Cr was added, the size of the colonies gradually became smaller and the time for appearance of colonies prolonged. The

cell size of CMBLY 3 is significantly decreased after Cr administration. After 12 hr of incubation the Cr treated cells were 2.5 to 3.75 pm in size, as against untreated cells (3.75 to 5.6 pm). After 24 hr the untreated cells ranged between 4.25 to 7.5 µm, while treated cells were only 2.5 to 5.0 µm in size. Cr treated cells usually occur as single cells with no or a few buds, whereas untreated cells have many buds i.e. they are multiplying.

Fifteen Cr-resistant isolates were checked for their resistance to other heavy metals (Table 1). The isolates were grown in YEPD medium containing various concentrations of lead acetate (0.5, 1, 2, 3, 4 $mgmL^{-1}$), cadmium chloride (100, 200, 300, 400, 500 μgmL^{-1}), copper sulphate (50, 100, 150, 200 $\mu grnL^{-1}$), mercuric chloride (50, 100, 150, 200 μgmL^{-1}) and cobalt chloride (100, 200, 300, 400 μgmL^{-1}). Lead was the least toxic. Four isolates (CMBLY 3, 36, 62 and 80) could grow at a concentration of 3 $mgmL^{-1}$ of lead, whereas seven isolates could grow on 2 $mgmL^{-1}$. Mercury was the most toxic metal studied. Three isolates (CMBLY 30, 61 and 64) could resist only 50 μgmL^{-1} Hg, whereas CMBLY 3, 10, 44, 46, 57 and 80 could tolerate 200 μgmL^{-1} of Hg in the medium. CMBLY 70 and 57 could grow in 400 μgmL^{-1} concentration of Cobalt, whereas CMBLY 31, 33, 36 and 50 could resist 300 μgmL^{-1} concentration of Cobalt. CMBLY 3, 31, 33, 46, 70, 57 and 80 could resist 200 μgm^{L-1} concentration of copper, but CMBLY 62 could grow up to 50 μgmL^{-1} of copper.

Figure 1 shows growth curves of CMBLY 3 in a medium with and without Cr^{+6}. It has a lag phase of 8 hr and log phase extending between 24 to 36 hr of incubation. In the presence of Cr (100 μgmL^{-1}), however, the lag phase is prolonged to 20 hr, and log phase drifts between 60 to 80 hr of incubation. The extended lag phase in the treated culture resulted in reduced number of cell divisions. Consequently, the cell number did not change significantly in the presence of Cr^{+6} until about 30 hr, whereas in the control culture, the number of cells/mL increased significantly within 4 to 6 hr of incubation. This is corroborated by studies of Ogawa et al. (1989), who have reported that $K_2Cr_2O_7$ inhibits DNA synthesis by interacting with DNA and/or inhibiting DNA polymerase activity. This apparently results in

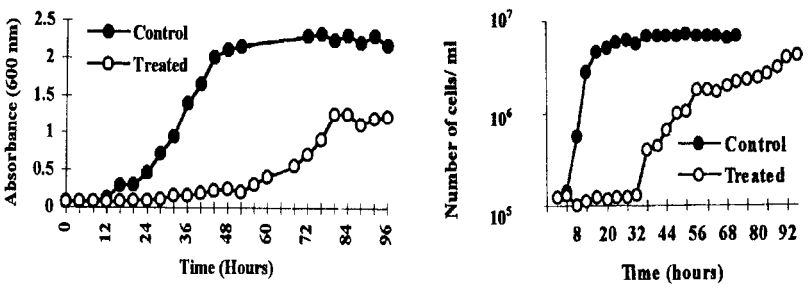

Figure 1. Effect of chromium on growth of yeast.

decreased cell division, and increased generation span. The morphology of yeast colonies and yeast cells was also affected in the presence of heavy metals. The surface of metal tolerant yeast colonies became convoluted, and the cells became smaller and generally more spherical (Brady et al. 1994) with irregular cell walls (Khan et al. 1991).

The isolate CMBLY-3 shows optimum growth at pH 4 and at 30°C (Fig. 2).

Twenty isolates were selected for analysis of their efficiency to remove Cr^{+6} from the medium (Table 2). CMBLY 3 and CMBLY 4 were the most efficient and could reduce 97% of Cr^{+6} from the ambient medium after 96 hr of incubation. Strain CMBLY 3 removes only 2% Cr after 48 hr and 26% after 72 hr of incubation. CMBLY 4 decontaminated 72% after 48 hr, and 87% after 72 hr of incubation. Isolate CMBLY 22 and CMBLY 27 reduced only 21% of Cr^{+6} after 96 hr of incubation.

Table 2. Percent reduction of Chromium VI in the medium by different yeast isolates.

	% reduction after		
Yeast isolates	**48 hr**	**72 hr**	**96 hr**
CMBLY 1	90	90	96
CMBLY 2	11	56	80
CMBLY 3	2	26	97
CMBLY 4	72	87	97
CMBLY 8	8	61	61
CMBLY 10	12	30	37
CMBLY 14	6	28	30
CMBLY 16	19	21	45
CMBLY 21	16	23	32
CMBLY 22	11	20	21
CMBLY 27	12	19	22
CMBLY 31	46	72	90
CMBLY 33	32	55	70
CMBLY 44	59	75	88
CMBLY 46	49	51	61
CMBLY 62	31	32	53
CMBLY 64	22	30	49
CMBLY 77	34	40	77
CMBLY 80	33	39	64

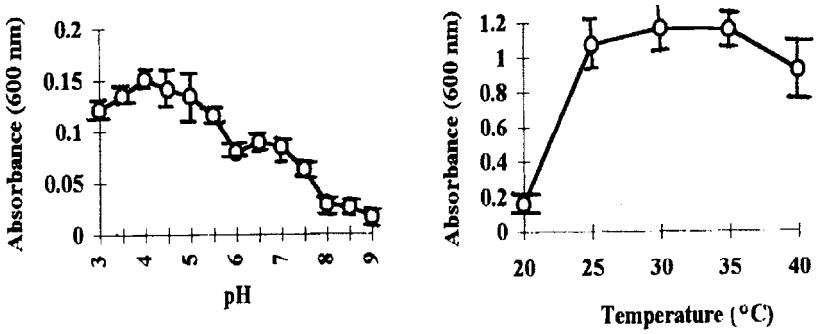

Figure 2. Optimum growth conditions of yeast.

There have been only a few reports on utilization of yeast for detoxification and recovery of heavy metals from contaminated environment (DeRome et al. 1991). Relatively slow growth and less efficiency of yeast as compared with bacteria may have been the reason. Moreover, Cr^{+6} resistant yeast have also been reported which do not reduce Cr^{+6} to Cr^{+3} species and also show very little accumulation of Cr^{+6} (Baldi 1990). The capability of yeast isolates to grow in the presence of toxic Cr^{+6} and to detoxify it, makes it an ideal candidate for waste water treatment and environmental clean up. Use of natural mechanisms like yeast bioassay would offer a potential alternative to conventional methods for detoxification of toxic metals.

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