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Silver Resistant Yeast Strains Isolated From Industrial Waste Water and Their Ability to Uptake Silver From The Medium

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Abstract.- Eleven silver resistant yeast strains (GCYAg1 - GCYAg11) were isolated from effluents collected from Nallah Daik (Sheikhupura, Pakistan), Rohi Nallah (Lahore, Pakistan) and Sitara Chemicals (Sheikhupura Road, Faisalabad, Pakistan) and were grown on YEPD medium. The minimum inhibitory concentration of Ag^{+} varied from 140 µg/ml (GCYAg1), 800 µg/ml (GCYAg3, 4 and 10). They could also tolerate nickel (2.0 to 5.0 mg/ml), cadmium (0.75 to 5.0 mg/ml), copper (1.0 to 6.0 mg/ml), lead (5.0 to 6.0 mg/ml) and chromium (0.5 to 3.5 mg/ml). The order of resistance to metals was $Cu^{2+} = Pb^{2+} > Ni^{2+} = Cd^{2+} > Cr^{6+} > Ag^{2+}$. The optimum temperature for growth of GCYAg3, 6, 7 and 9 was 30°C, for GCYAg2 and 11 was 37°C, and for GCYAg1, 4, 5, 8 and 10 was 45°C. The optimum pH was 5.0 for GCYAg6, 5.5 for GCYAg7, 6.0 for GCYAg2, 7.0 for GCYAg9 and 11, 8.0 for GCYAg8, 9.0 for GCYAg4 and 10, and 9.5 for GCYAg1 and 5. The isolates were evaluated for their efficiency of removing silver from the medium. They could remove 60-97% silver from the medium. The SDS-PAGE analysis of total proteins indicated formation of new bands in silver treated samples, but some disappeared, thus showing their adaptation to stress conditions of isolates. Results of this work indicate the potential of the yeast isolates for bioremediation of industrial wastewater of heavy metals.

Key words: Bioremediation, heavy metal toxicity, growth curves, metal uptake.

INTRODUCTION

Today the problem of environmental pollution and detoxification of industrial wastes has become a matter of increasing concern. Industrial wastes containing heavy metals and organic pollutants enter our water resources directly or indirectly through food chain. Silver is one of the heavy metals that reach the general population through drinking water and food and hence affect human and animal life. Dental medical uses of silver and occupational exposure to silver and its compounds is also reported (Jongeneelen, 1992). Abnormalities of Vitamin B12, folic acid and thyroid have been reported from a silver-reclaiming factory (Blanc *et al.,* 1985). It has also been reported to provoke hepatic, renal and neurological tissue toxicity (Maitre *et al.,* 2002).

The physical and chemical methods to decontaminate industrial waste water of toxic compounds inclusive of heavy metals are not only expensive; they are also not risk free. One may end up adding more chemicals in the environment in a bid to precipitate or filter silver. Bioremediation is, on the other hand, potentially more effective, least hazardous and cost effective. Microorganisms can be used to remove toxic metals and metalloids from contaminated soil, waters and waste streams (White *et al.,* 1997). Several species of bacteria and yeast are capable of accumulating metal ions up to concentrations several orders of magnitude higher than the background environmental concentration (Khattar *et al.,* 1999; Kratochvil and Volesky, 1998; Krauter *et al.,* 1996). It is well recognized that microorganism have high affinity for metals and can accumulate both heavy and toxic metals by a variety of mechanisms (Silver, 1991; Simmons *et al.,* 1995; Suttleworth and Unz, 1993). The main objective of the present study was to isolate silver resistant yeast from the industrial wastewater and assess their ability to uptake the metal from the medium with an ultimate objective of using these isolates for environmental clean up.

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MATERIALS AND METHODS

Sampling sites

Industrial wastewater and soil samples were collected in sterilized glass bottles from Nallah Daik (Ittehad Chemicals Ltd., Sheikhupura), Rohi Nallah (Glaxo Welcome Ltd., Khass (Pvt) Ltd., Lahore) and Sitara Chemicals Ltd. Sheikhupura Road, Faisalabad. The temperatures and pH of the samples were also recorded at the time of sampling.

Isolation and purification of Ag-resistant yeast

Fifteen µl of waste water was spread on YEPD agar medium containing 50 µg of silver and incubated at 30°C for 48 hours. Cells from colonies were observed under microscope and the process was repeated three time to get pure cultures and were labelled as GCYAg-1 to 11. These colonies were then streaked on fresh medium containing 100 µg of silver. In this way every time the silver concentration was increased by 50 µg/ml and then the cells transferred to higher concentration, until the yeast stopped growing. The last concentration of Ag^{+} used was taken as minimum inhibitory concentration (MIC).

YEPD agar medium (1.0g yeast extract, 0.5g peptone, 0.02g D-Glucose dissolved in 100 mL of distilled water, pH adjusted at 7.6 followed by the addition of 1.5g of agar, autoclaved at 121°C at 151b pressure for 15-20 minutes) was poured in sterilized Petri plates. About 15 µl of each of the antibiotics Gentamycin (0.3%) and Chloramphenicol (0.5%) were added in 100 ml of autoclaved medium.

Cross heavy metal resistance

The isolated Ag-resistant yeast strains were checked for their resistance to Cr^{6+}, Cd^{2+}, Co^{2+}, and Cu^{2+}. For this purpose, YEPD medium was prepared with 50 mg/ml, each of $K_2Cr_2O_7$, $CdCl_2$, $CuSO_4$, Pb $(CH_3COO)_2$ and Ni $(CH_3COO)_2$. The YEPD agar media with metal ions solutions were autoclaved in separate flasks. Then each metal solution was mixed thoroughly with 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. The concentration of these heavy metals in agar plates were increased gradually from 50 mg/ml by adding different concentrations of these salt solutions in agar pates.

Morphological characterization of the yeast isolate

In order to check colony morphology of yeast, Yeast morphology agar (3.5 g) was dissolved in 100 ml distilled water, autoclaved and poured into sterilized Petri plates. After streaking, the plates were incubated at 30°C for 48 hours. Then following characteristics of yeast colonies were noted; color of colony, shape and surface of colonies, size of colonies and texture and margins of colonies. For cell morphology (cell size and cell shape), cell suspension was made in a drop of distilled water on a clean side and then it was observed under a microscope.

For study of mycelia, the yeast colonies were streaked lightly on autoclaved YEPD agar medium as a cross. At the center of the cross a sterile cover slip was placed. Plates were incubated for 7 days at 30°C. These were then examined under microscope to observe the growth of hyphae.

Sporulation test

For studying sporulation, four types of media *viz*., GordKowa agar medium (0.1 g glucose,1.0g peptone, 0.5g NaCI and 2.0g agar in 100 mL distilled water), aqueous agar (2g aqueous agar in 100 ml distilled water), Malt yeast glucose peptone agar medium (0.3g Malt extract, 0.3g yeast extract, 1.0g glucose, 0.5g peptone and 2.0g agar in 100 mL distilled water), and Malt extract agar (4.0g malt - extract, 0.5g yeast extract and 1.5g agar in 100 mL distilled water) were prepared, autoclaved, poured in sterilized Petri Plates and incubated at 30°C to check for any contamination.

The pure cultures of yeast isolates were streaked on the respective media and incubated at 30°C. After one weak, yeast colony was removed and a smear was prepared and dried at room temperature. Then it was fixed and spore staining was done using Malachite green (5% aqueous). The slide was steamed on flame for 5 minutes and rinsed with distilled water. Then safranine (0.5% aqueous) was added for 30 seconds and washed with distilled water. Smear was observed under microscope. Spores were stained green, while the cells stained pink.

Biochemical characterization

All yeast isolates were tested for nitrate reduction, growth at 5% of glucose and 10% of NaCI, ester production, growth at 50% glucose medium, starch production, urease activity, Diazonium Blue B Test, tolerance of 1.0% acetic acid, assimilation of carbon compounds and oxidation fermentationt. All these biochemical tests were performed as described in Collee *et al.* (1998) and Kreger-Van-Rij (1987).

Determination of optimum growth conditions for yeast isolates

For determination of optimum growth conditions for each isolate, two parameters *i.e.* pH and temperature were considered.

For determination of optimum temperature, 5 ml YEPD broth in five sets, each with three test tubes was inoculated with freshly prepared yeast culture and incubated at five different temperatures *viz.* 25°C, 30°C, 35°C, 40°C and 45°C for 20 hours to check the growth of each yeast isolate. Optical density of each tube, as a measure of growth, was noted at 600 nm wavelength. The graph was plotted using the data, temperature (along X-axis) and respective absorbance value (along Y-axis). The peaks of graph indicated the optimum temperature.

For determination of optimum pH, 5 ml YEPD broth was taken in 13 sets, each with three test tubes in which pH values were adjusted at 4.0,4.5,5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. All the tubes were autoc1aved, inoculated with fresh culture of yeast isolates and incubated in shaking water bath for 20 hours to check the growth of each yeast isolate. Optical density was noted at 600 nm wavelengths and graph was plotted taking pH value (along X-axis) and their respective absorbance (along Y-axis). With the help of this graph, the optimum pH of each yeast isolate was determined.

Preparation of growth curves

For preparation of growth curve, 100 ml YEPD broth in two (250 ml) flasks, one as control and one for treatment, were used. In treatment flasks, 200 µl of stock solution of silver nitrate (50 mg/ml) was added to make final concentration of 5 µg/ml. The control as well as treatment flasks were inoculated with 1 ml of fresh yeast culture (log phase) incubated at 30°C in shaking water bath. Two ml medium was taken out from each flask after every hour for two days. O.D. of cultures was taken at 600 nm. Then a graph was plotted taking time interval along X-axis and respective absorbance values along Y-axis.

Determination of silver uptake by the isolates

To determine and evaluate the ability of yeast isolates to accumulate silver ions from their environment, silver nitrate solution was added to the medium at the concentration of 100 µg/ml. Media were inoculated with a freshly grown (overnight) yeast culture. Small samples (5ml) of the medium were taken after regular intervals of 12 hours, culture was spun down at 3000 rpm and the concentration of the metal ions was estimated in the supernatant with the help of atomic absorption spectrophotometer.

A control was maintained without inoculation but with the same concentration of metal as was added in the yeast culture. These estimations were done in triplicate.

PAGE analysis of protein

The proteins of yeast strains were isolated according to Horvath and Riezman (1994), estimated according to Lowry *et al.* (1951) and electrophoresed according to Laemmli (1970). The polyacrylamide gel (12.5%) was run at 50 volts for 4 hours, stained with coomassie blue and then destained with destaining solution (150 ml methanol and 50 ml glacial acetic acid in 300 ml distilled water) on an orbital shaker for 30 minutes.

RESULTS

Silver resistant yeast isolates

Two yeast strains (GCY Ag-7 and 9) were isolated from Nallah Daik (Sheikhpura Road), showing MIC of 155 and 700 µg/ml. Eight isolates from Rohi Nallah (Lahore Multan Road) (GCY Ag-1-6,10,11) showed MIC's ranging between 140-800 µg/ml, whereas one isolated (GCYAg-8) from effluents of Sitara Chemical had MIC of 750 µg/ml.

Table I shows cross resistance of heavy metals against yeast isolated selected on Ag^+ containing medium.

Table I: MICs of different heavy metals against yeast isolates from industrial effluents.

Strain No.	Ag^{+} (mg/ml)	Ni^{+2} (mg/ml)	Cd^{+2} (mg/ml)	Cu^{+} (mg/ml)	Pb^{+2} (mg/ml)	Cr^{+6} (mg/ml)
GCY Ag-1	0.14	2.5	4.5	6.0	6.0	0.5
GCY Ag-2	0.65	5.0	1.0	6.0	5.25	3.0
GCY Ag-3	0.8	5.0	5.0	5.25	6.0	3.0
GCY Ag-4	0.8	2.5	4.5	5.0	6.0	3.0
GCY Ag-5	0.75	4.0	4.00	5.5	5.25	2.5
GCY Ag-6	0.2	4.75	4.5	5.0	6.0	1.5
GCY Ag-7	0.155	-	4.25	1.5	5.25	3.5
GCY Ag-8	0.75	4.75	3.75	5.0	-	2.75
GCY Ag-9	0.7	4.0	4.75	1.5	5.25	3.0
GCY Ag-10	0.8	4.0	5.0	2.0	5.5	2.0
GCY Ag-11	0.65	4.5	4.5	3.25	5.25	

Eleven Ag^{+} resistant yeast strains selected on YEPD medium containing 10.0 µg Ag^{+}/ml, were isolated from effluent samples. Their order of tolerance in descending order against different metal is as follows;

Silver: GCYAg-3, 4, 10> GCYAg-5=8> GCYAg-9> GCYAg-2, 11> GCYAg-6> GCYAg-7> GCYAg-1.

Nickel: GCYAg-2=3> GCYAg-6=8> GCYAg-11> GCYAg-5=9=10≥ GCYAg-1=4

Cadmium: GCYAg-3=10> GCYAg-9> GCYAg-1=4=6=11> GCYAg-7> GCYAg-5> GCYAg-8> GCYAg-2

Copper: GCYAg-1=2> GCYAg-5> GCYAg-3> GCYAg-4=6=8> GCYAg-11> GCYAg-10> GCYAg-7=9

Lead: GCYAg-1=3=4=6> GCYAg-10> GCYAg-2=5=7=9=11.

Chromium: GCYAg-8> GCYAg-2=3=4=10> GCYAg-9> GCYAg-5> GCYAg-11> GCYAg-6> GCYAg-1

Isolates with high MIC for Ag^{+} have high MICs for other heavy metals too. For example GCYAg-3 and 4 have MIC of 0.8 mg/ml for Ag^{+}, 5.0 mg/ml for Ni^{2+}, Cd^{2+} and Cu^{2+}, 6.0 mg/ml for Pb^{2+} and 3.0 mg/ml for Cr^{6+}.

Growth conditions of isolates

Most of the yeast isolates showed optimum growth' at 30°C (GCYAg-3, 6, 7, 9) whereas GCYAg-2, GCYAg-11 showed maximum growth at 37°C, and GCYAg-1, 4, 5, 8, 10 at 45° C. The optimum pH was 5.0 for GCY Ag-6, 5.5 for GCY Ag-7, 6.0 for GCY Ag-2, 7.0 for GCYAg-9 and 11, 8.0 for GCYAg-8, 9.0 for GCYAg-4 and l0, 9.5 for GCYAg-I and GC5.

Growth curves

Sigmoid growth curve with well defined lag, log and stationary phases were obtained with short lag phase of 4-14 hours followed by 14-44 hours of log phase. Presence of silver in the media greatly affected the growth pattern of the yeast. Prolonged lag phase of 6-20 hours was followed by log phase with lesser growth as compared with the log phase of controls.

Silver processing efficiency of yeast isolate

In order to determine the silver processing abilities of isolated yeasts, inoculum of 100 µl (5 µg/ml) of silver solution was used in 50ml media. The reducing ability of the isolate was observed after the incubation for 48 hours. The yeast strains GCY Ag4, 5, 7, 8 and 9 showed maximum processing ability, which is more than 90%. But yeast strains GCYAg-3, 10 and 2 showed reducing ability ranging between 60% and 70%, while in the case of GCY Ag-I,6 and 11 the silver processing ability was more than 80% (Table II).

SDS page analysis

The SDS-PAGE analysis of total proteins indicated formation of new bands (GCYAg4, 5, 7, 9, 11) in silver treated samples, but in some isolates (GCYAg2,8) protein bands disappeared. Similar trend was observed in yeast strains GCYAg1, 3, 6, 10. Appearance and disappearance of the bands are indicative of expression of stressful condition and induction of protein synthesis (Figs. 1, 2, 3).

Table II: Amounts of Ag^+ removed (%) from 50 ml culture medium containing 5 µg/ml of Ag^+ and inoculated with 100 µl of log phase culture of yeast isolates from industrial wastewater. the metal estimations were done after 48 hours of incubation.

No.	Yeast Strains	Percentage reduction of metal from the medium
1	GCYAg-1	82.9%
2	GCYAg-2	73.6%
3	GCYAg-3	61.1%
4	GCYAg-4	94.9%
5	GCYAg-5	91.90%
6	GCYAg-6	85.8%
7	GCYAg-7	90.5%
8	GCYAg-8	97%
9	GCYAg-9	95%
10	GCYAg-10	62.8%
11	GCYAg-11	86.2%

DISCUSSION

Eleven Ag^+ yeast strains were isolated from Nallah Daik, Rohi Nallah and Sitara Chemicals' effluents. Metal resistant of most yeast strains like *Saccharromyces cerevisiae/ Candida and Pichia* have seen reported from other laboratories (Rapport and Muter, 1995; Raspor *et al.* 2000; Ksheminka *et al.,* 2002). Yang and Pon (2003) studied the silver resistant yeast and proved that most of the toxic metal ions are capable of inducing oxidative stress on cells through the mitochondria and respiratory chain. Mitochondria may contribute to and be target of metal toxicity.

Elevation of the metal concentration in the medium resulted in delayed growth and diminished colony size of all isolates. Brady *et al.* (1994) have also reported reduction in yeast cell size and convolution of cells on exposure to heavy metals. It has been shown that resistance often occurs for a range of metal ions rather for a specific metal only (Yasmin and Husnain, 1991; Schmidt and Schlegel, 1994; Mergeay, 1991; Dressler *et al.,* 1991). Different mechanisms may be present for heavy metal resistance in microbes, for example intracellular sequestration, permeability barrier, extracellular sequestration and extracellular detoxification (Choi *et al.,* 1995). The cell walls of microorganism play important role for metal binding (Avery and Tobin, 1992, 1993; Yazgan and Ozcengiz, 1994;. Simmons *et al.,* 1995; Volesky and May-Philips, 1995). The strains resistant to heavy metals may be due to the presence of resistant genes like SMF1, SMF2 and SMF3 in yeast (Supek *et al.,* 1997).

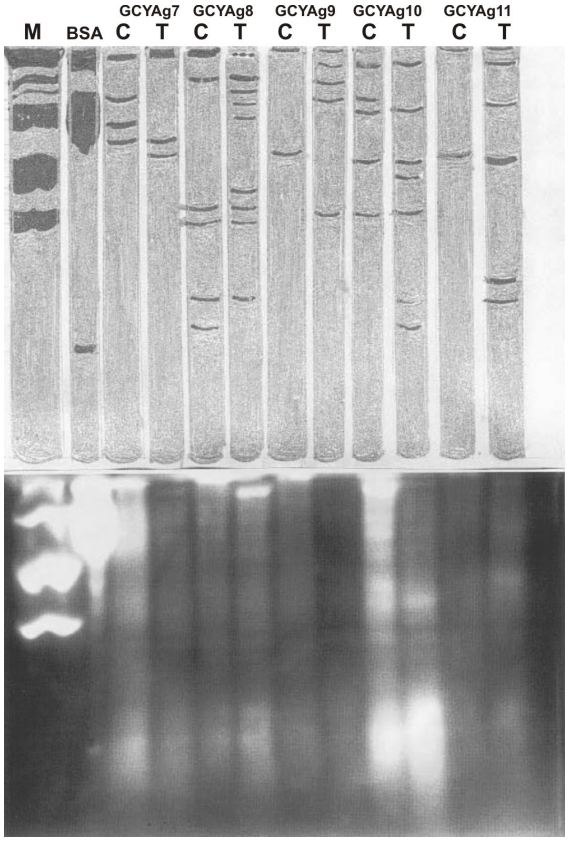

Fig. 1. PAGE pattern of total proteins of Ag^+ resistant yeast (GCYAg 6-8, 10, 11) isolated from soil samples and industrial waste waters. C, control; T, treatment with Ag^+.
M, marker; BSA, bovine serum albumin; GCYAg6 (Control) four bands, (Treated) 3 bands, new as of 80 Kd, bands of 97 Kd, 100 Kd disappeared; GCYAg7 (Control) six bands; (Treated) 9 bands new were of 66 Kd, 95 Kd, 98 Kd, 100 Kd and of 45 Kd disappeared; GCYAg8 (Control) 2 bands; (Treated) 5 five bands, new were of 60 Kd, 98 Kd, 100 Kd, 120 Kd; GCYAg10 (Control), six bands; (Treated), 7 bands new were of 40 Kd, 45 Kd, 60 Kd and bands of 97, 180 Kd disappeared; GCYAg11 (Control) 2 bands, (Treated) six bands, new were of 40 Kd, 45 Kd, 97 Kd and 180 Kd.

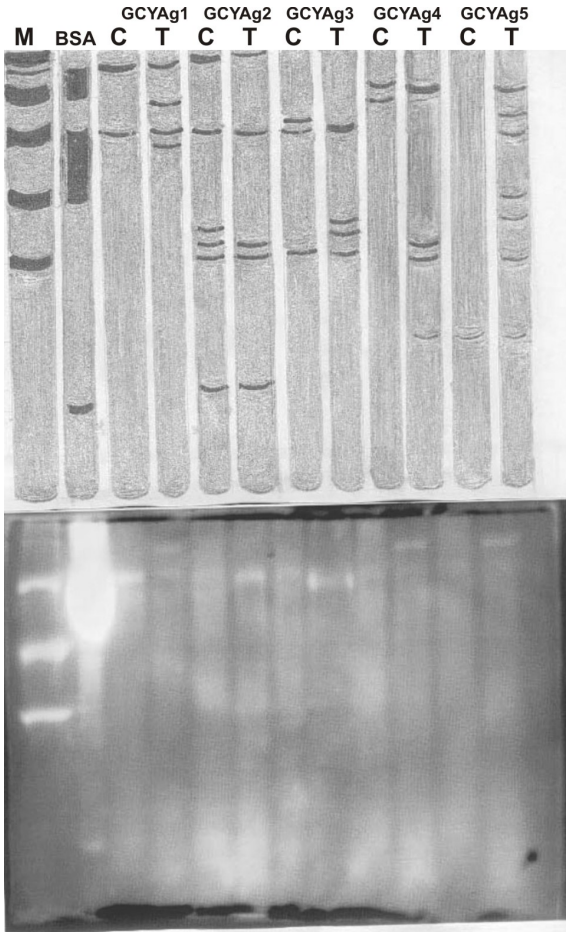

Fig. 2. PAGE pattern of total proteins of Ag^+ resistant yeast (GCYAg 6-8, 10, 11) isolated from soil samples and ndustrial waste waters. C, control; T, treatment with Ag^+.
M, marker; BSA, bovine serum albumin; GCYAg1 (Control) two bands, (Treated) 4 bands, 2 new; GCYAg2 (Control) six bands; (Treated) 5 bands new were of 50 disappeared; GCYAg3 (Control) 3 bands; (Treated) 4 five bands, new were of 58 Kd, 64 Kd and 100 Kd disappeared; GCYAg4 (Control), 2 bands; (Treated), 4 bands new were of 45 Kd, 50 Kd and 55; GCYAg5 (Control) 1 band, (Treated) 7 bands, new were of 45 Kd, 50 Kd, 66 Kd, 90 Kd and 100 Kd.

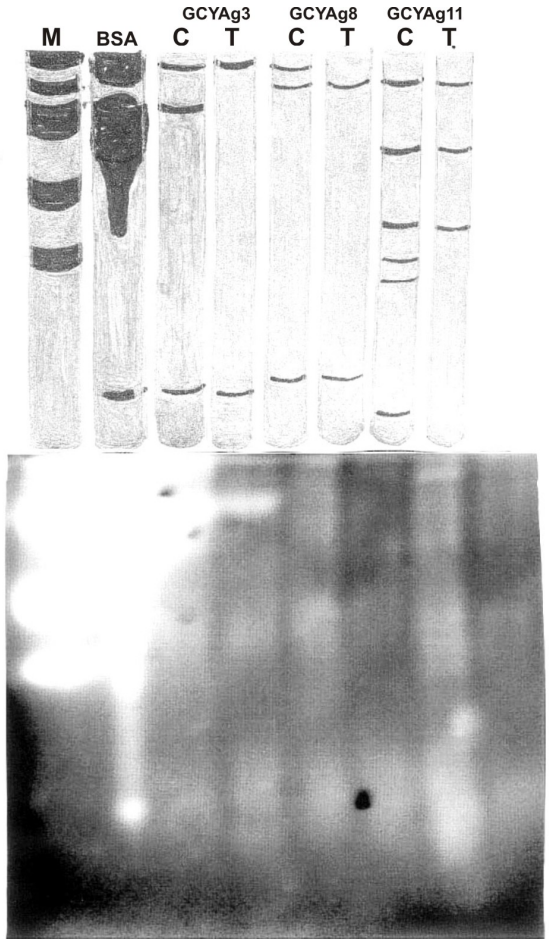

Fig. 3. PAGE pattern of total proteins of Ag^+ resistant yeast (GCYAg 6-8, 10, 11) isolated from soil samples and ndustrial waste waters. C, control; T, treatment with Ag^+.
M, marker; BSA, bovine serum albumin; GCYAg3 (Control) 3 bands, (Treated) 2 bands, 1 disappeared; GCYAg8 (Control) 3 bands; (Treated) 2 bands 1 disappeared; GCYAg11 (Control) 6 bands, (Treated) 3 bands, 3 disappeared.

Accumulation of hazardous elements by yeast cells is known for several years (Rossi, 1976; Wolf *et al.,* 1982; Cibulka *et al.,* 1992). Metal uptake is both passive and active process depending on the viability of biomass. This process is influenced by a number of environmental and experimental factors such as addition of an energy source, intrinsic biochemical structural and genetic properties of the yeast cell, pH, temperature, and the presence of additional ions, amongst others (Starling and Rass, 1991; Avery and Tobin, 1992, 1993; Dancis *et al.,* 1994; Volesky, 1994; Bengtsson *et al.,* 1995).

Optimum temperature for the growth of GCYAg-3, 6 and 9 was 30°C, also reported by Amanchukwu *et al.* (1989), whereas for GCYAg-2

and 11 was 37°C. Certain yeast strains were capable of growth at temperatures higher than 45°C (Kereger-van-Rij, 1987). The optimum pH 9, 11 for GCYAg6 is 5.0, GCYAg-7 is 5.5, GCYAg-2 is pH 6. GCYAg have growth on neutral pH. Remaining stains grow properly in basic pH from 8.0 to 9.5. All these results are in accordance with previous data (Lee *et al.,* 1993; Gosh and Banegea, 1986; Amonchukwa *et. al.,* 1989).

All the four isolates were very efficient in silver removal from medium growth in optimum conditions for 48 hours. As they can remove silver 61% to 97%. Yazgan *et al.* (1993) reported that yeast *(Kluyveromyces marxianus)* could remove 90% of silver and 65% of cadmium from growth medium.

The comparison of protein bands in control and silver treated samples were observed. New bands appeared and some bands became darker and thicker than control. Goyer (1986) has reported that certain toxic chemicals enhance the expression of some special genes whereas others would inhibit the normal expression. Furthermore, *K. marxianus* synthesized more proteins on induction by silver or cadmium (Yazgam *et al.,* 1993)

In the present study the silver resistant yeast isolates surviving in the presence of high concentration of silver could help in removal of heavy metals from the environment. Development of strategy for better use of those yeast strains for the removal of silver from the industrial effluents can help in environmental cleanup. .

REFERENCES

AMANCHUKW, S.C., OBAFEMI, A. AND OKPOKWASILE, G.C., 1989. Single cell protein production by *Schizosaccharomyces pombe*, isolated from palm wine using, hydrocarbon feed stocks. *Folia Microbiol.*, **34**: 113-119.

AVERY, S.V. AND TOBIN, J.M., 1992. Mechanism of Sr uptake by laboratory and brewing strains of *Saccharomyces cerevisiae. Appl. environ. Microbiol.,* **58**: 3883-3889.

BENGTSSON, L., JOHANSSON, B., HACKETT, T., McHALE, L. AND McHALE, A., 1995. Studies on the biosorption of uranium by *Talaromyces emersonii* VBS 81470 biomass. *Appl. Microbiol. Biotechnol.*, **42**: 807-811.

BLANC, P., HOGAN, M., MALLIN, K., HRYHORCZUK, D., HESSL, S. AND BERNARD, B., 1985. cyanide intoxication among silver-reclaiming workers. *J. Am. med. Assoc,* **253**: 367-71.

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

CHOI, S.Y., BAEK, E.M. AND LEE, S.Y., 1995. A cDNA differentially expressed by cadmium stress in *Arabidopsis. Plant Physiol.,* **108**: 849.

CIBULKA, J., TURECKI, T., MIHOLOUA, D., MADDER, P., SZAKOVA, J. AND BRABEC, M., 1992. Cadmium, lead and mercury levels in feeding yeast produced in Czechoslovakia. *Sci. Total Environm.,* **114**: 73-86

COLLEE, J.G., DUGUID, J.P., FRASER, A.G. AND MARMOIN, B.P., 1998. *Mackie and MacCartney Practical medical microbiology,* vol. 2. Churchill Livingstone, Longman Group.

DANCIS, A., HAILE, D., YUAN, D.S. AND KLAUSNER, R.D., 1994. The *Saccharomyces cerevisae* copper transport protein. Biochemical characterization regulation by copper and psychological role in copper uptake. *J. biol. Chem.,* **269**: 25660-25667.

DRESSLER, C., KUES, U., NIES, D. AND FRIEDRICH, B., 1991. Determination encoding resistance to several heavy metals in newly isolated copper-resistant bacteria. *Appl. environ. Microbiol.,* **57**: 3079-3085.

GOSH, B.B. AND BANERJEE, A.R., 1986. Production of methioine and glutamic acid from n – alkanes by *Serratia marcescens varkiliensis. Folia Microbiol.,* **31**: 106-112.

GOYER, R.A., 1986. Toxic effects of metals. In: *Casarett and Doull's Toxicology. The basic science of poisons* (eds. C.D. Klaassen, M.O. Amdur and J Doull), 3rd edition, pp 592. MacMillan Publishing Company, New York.

HADDAD, L.M. AND WINCHESTER, J.F., 1983. *Clinical management of poisoning and drug overdosage*. pp. 662. W.B. Sauanders, Philadelphia.

HUNTER, D., 1983. *Encyclopaedia of occupational health and safety.* vol. 2, pp. 2047-2048.

JONGENEELEN, F.J.,1992. *Occupational exposure limits*.W.B.Saunders, Philadelphia.

KHATTAR, J. I. S., SARMA, T. A. AND SINGH, D. P., 1999. Removal of chromium ions by agar immobilized cells of the cyanobacterium *Anacystis nidulans* in a continuous flow bioreactor. *Enzyme Microbial. Technol*., **25:** 564–568

KRATOCHVIL, D. AND VOLESKY, B., 1998. Advances in the biosorption of heavy metals. *TIBTech*., **16:** 291–296.

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

KREGER-VAN RIJ, N.J.W., 1987. In: *The yeasts, a taxonomic study* (eds. N.J.W. Kreger van Rij and Groningen), pp.. 77-84. Elsevier Science Publisher, B.V. Amstredam, The Netherlands.

KSHEMINSKA, H., JAGLARZ A., FEDOROVYCH 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 (IV) and the influence of riboflavin on Cr tolerance. *Microbiol Res.,* **158**: 59-67.

LOWRY, O.H, ROSEBROUGH, N.J. FARR, A. AND RANDAL, R.J 1951. Protein measurement with Folin Phenol Reagent. *J. biol. Chem*., **193**: 265-275.

LINDQUIST, S. AND CRAIG, E. A., 1988. The heat-shock proteins. *Annu. Rev. Genet*., **22:** 631–677.

LEE, C., YAMAKAWA, T. AND KODAMA, T., 1993. Rapid growth of thermotolerant yeast on palm oil. *Wld. J. Microbiol. Biotechnol.,* **9**: 187-190.

MAITRE, S., JABER, K., PEROL, J.L., GUY, C. AND CAMBAZARD, F., 2002. Increased serum and urinary levels of silver during treatment with topical silver sulfadiazine. *Ann. Dermatol. Venereol*., **129**: 217-219.

MERGEAY, M.L., 1991. Towards an understanding of the genetics of bacterial metal resistance. *TIBTech.,* **8**: 551-567.

RAPOPORT, A. I. AND MUTER, O. A., 1995. Biosorption of hexavalent chromium by yeast. *Process Biochem.,* **30:** 145–149.

RASPOR, P., BATIC, M., JAMNIK, P., JOSIC, D., MILACIC, R., PAS, M., RECEK, M., REZIC-DEREANI, V. AND SKRT, M., 2000. The influence of chromium compounds on yeast physiology (a review). *Acta Microbiol. Immunol. Hung.,* **47:** 143–173.

ROSI, V., 1976. Proteins from petrol. *Bull. Lab. Chim. Provin.,* **27**: 161-173.

SCHMIDT, T. AND SCHLEGEL, H.G., 1994. Combined Nickel-Cobalt cadmium resistance encoded by the ncc locus of *Alcaligenes xylosoxidans* 31A. *J. Bact.,* **176**: 7045-7054.

SILVER, S., 1991. Bacterial heavy metal resistance systems and possibility of bioremediation. In: *Biotechnology, bridging research and application*, pp. 265-287. Kluwer, Academic Publisher, London.

SIMMONS, P., TOBIN, J.J. AND SINGLETON, I., 1995. Consideration on the rise of commercially available yeast biomass for the treatment of metal containing effluents. *J. ind. Microbiol.,* **14**: 240-246.

STARLING, A.P. AND ROSS, I.S., 1991. Uptake of zinc by *Penicillium notatum. Mycol. Res.,* **95**: 712-714.

SUPEK, F., SUPEKOVA, L., NELSON, HA. AND NELSON, N., 1997. Function of metal-ion homeostasis in cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain functions. *J. exp. Biol*., **200**: 321-330.

SUTTLEWORTH, K.L. AND UNZ, R.F., 1993. Sorption of heavy metals to the filamentous bacterium tholthrix strain A1. *Appl. environ. Microbiol*., **59**: 1274-1282.

VOLESKY, B., 1994. Advance in biosorption of metals selection of biomass type. *FEMS Microbiol. Rev.,* **14**: 291-302

VOLESKY, B. AND MAY-PHILLIPS, H. A., 1995. Biosorption of heavy metals by *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol*., **42:** 797–806.

WHITE, C., SAYER, J.A. AND GADD, G.M., 1997. Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiol. Rev.,* **20:** 503–516.

WOLF, A., HRUBY, S., HRIVNAK, D. AND SVABOVA, M., 1982. Hygienic evaluation of some untraditional protein sources. *Ceskoslov. Hyg.,* **27**: 108-111.

YANG, H.C. AND PON, L.A., 2003. Toxicity of metal ions used in dental alloys: a study in the yeast *Saccharomyces cerevisiae. Drug Chem. Toxicol.*, **26**: 75-85.

YASMIN, S. AND HUSNAIN, S., 1991. Cobalt resistant and *Psedumonas* from industrial effluent. *Punjab Univ. J. Zool.,* **6**: 43-48.

YAZGAN, A. AND OZCENGIZ, G., 1994. Subcellular distribution of accumulated heavy metals in *Saccharomyces cerevisiae* and *Kluyveromyces marxians. Biotechnol. Lett.,* **16**: 871-874.

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