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Metal resistance and uptake by *Trichosporon asahii* and *Pichia kudriavzevii* isolated from industrial effluents

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Abstract: Metal-resistant yeast strains, *Trichosporon asahii* and *Pichia kudriavzevii*, were grown-well onto YPD medium at 37°C (pH 6) and 30°C (pH 7), respectively. Tolerance values determined in *T. asahii* were 35 mM (Pb), 33 mM (Cu), 30 mM (As) and 10 mM (Cd) while *P. kudriavzevii* resisted up to 31 mM (Pb), 27 mM (Cu), 15 mM (Cd) and 12 mM (As). Yeasts grown in Minimal Salt Medium (MSM) were treated separately with metal challenge (100 mg/L) for 2 days. *T. asahii* showed elevated glutathione (GSH) level with Cd (83.06), As (81.87), Pb (66.88) and Cu (56.19) mM/g which was 70 (Cu), 69.87 (Pb), 56.47 (As) and 52.76 (Cd) in *P. kudriavzevii* as compared to the control. The glutathione (GSH): glutathione disulfide (GSSG) ratio was decreased with all treated heavy-metals except Cd in *T. asahii* and increased with Cu and Pb in *P. kudriavzevii*. *T. asahii* could remove 78% (Cd), 72% (As), 85% (Cu) and 94.5% (Pb) from the medium after 12 days while was able to uptake 44.8, 41, 62 and 72 mg/g Cd, As, Cu and Pb, respectively. Likewise, *P. kudriavzevii* was able to remove 61% (Cd), 62% (As), 61% (Cu) and 87% (Pb) after 12 days of incubation and also showed capacity to uptake 36.8, 48, 40 and 57 mg/g Cd, As, Cu and Pb, respectively. Total protein profiling of yeasts revealed marked differences in banding pattern due to increased oxidation under metal stressed conditions. High metal uptake ability makes *T. asahii* and *P. kudriavzevii* potential candidates to remove metals from the environment.

Introduction

Heavy metal pollutants in the environment can enter the trophic cycle and pose a serious threat to human health through acute and chronic exposure in the food chain. Technology to date offers many techniques for abatement of metal pollution from industrial waste streams. Biosorption with microbial biomass is one strategy that is perceived as cost effective and suitable low concentrations of heavy metals removal (Yan and Viraraghavan 2003, He et al. 2011, Huang et al. 2014, Celebi et al. 2016, Uzun and Sahan 2017). Bioaccumulation consists of independent extracellular adsorption of metal ions by surface complexation, ion exchange or electrostatic interaction and active, energy-dependent uptake of metal ions which leads to intracellular accumulation (Vargas-García et al. 2012).

Yeast cells have been widely used for bioremediation due to inexpensiveness, easy availability and high biosorption potential attributed to large cell size (Shakya et al. 2015). Yeast can remove metal ions either by passive adsorption on cell surface or active intracellular accumulation. Exact mechanism of metal biosorption is not fully understood and found dependent on various parameters such as type of biomass, properties of metal ions, presence of co-metal ions, temperature and pH of the medium (Febrianto et al. 2009, Khan et al. 2016).

In general, metals generate reactive oxygen species (ROS), which can contribute to the toxicity by impairing cellular metabolism. In response, antioxidant enzyme systems in eukaryotic microbes can be induced. The glutathione (GSH)-glutathione disulfide (GSSG), the most abundant redox system in fungi and yeasts, plays a fundamental role in cell homeostasis and is considered as an index of oxidative damage (Valko et al. 2007). The major line of defense for the protection of cells from oxidative stress is GSH and GSH-linked metabolism (Dickinson and Forman 2002). GSH is involved in many biological processes including DNA synthesis, inactivation of drugs and detoxification of xenobiotic molecules, carcinogens, and ROS. GSH also performs various functions in the protection, enzymatic catalysis, and transport of amino acids (Penninckx 2002, Kim et al. 2005). GSH modulates protein structure either directly or indirectly by effecting sulfhydryl groups of protein (Cotgreave and Gerdes 1998, Klatt and Lamas 2000). Metallothioneins (MTs), cysteine-rich low molecular weight proteins, have also been reported to sequester some metal ion in the cytoplasm, thus mitigating the damage (Yang et al. 2015).

The objective of the present study was to isolate multiple metal ions resistant yeast isolates capable to remove such metal ions from the industrial wastewater. Furthermore, yeast isolates were tested for their capacity of metal removal by adsorption and bioaccumulation. Their resistance to toxic metals was characterized by analyzing GSH and non-protein thiols in the cell biomass.

Materials and Methods

Sample collection and isolation of yeasts

Samples of industrial effluents were collected in sterile containers from Sheikhupura, near Lahore in the Punjab Province, Pakistan. Aliquots were spread onto yeast-peptone--dextrose (YPD) plates (2.0% glucose, 1.0% yeast extract, 2.0% bacto-peptone (2%), 2.0%; pH 6.5). Minimal salt medium (MSM) (0.086% $CaCl_2$, 0.02% $FeSO_4$, 0.15% K_2HPO_4, 0.1% KH_2PO_4, 0.1% $MgSO_4$, 1% $(NH_4)_2SO_4$ and 1% glucose) was used and incubated at 120 rev/min at 30°C for 24 h. The pH of the medium was adjusted to 7.0–7.2. For metals, stocks solutions of $CdCl_2$, $NaAsO_2$, $CuSO_4$, and $Pb(NO_3)_2$ were prepared in deionized water. Cultures grown for 24 h received the metals for 2 days of contact and highest resistance strains were selected for further experiments.

Growth conditions

The temperature and pH optima were tested for the two isolates. The strains were cultured in (YPD) liquid medium (pH 7) at 20°C, 30°C, 37°C, 45°C and 55°C. For the pH experiment, cultures (100 ml YPD in 250 shake flasks) were adjusted to pH 5, 6, 7, 8, 9 and 10 and incubated at the optimum temperature. For growth pattern, yeast isolates were grown in MS broth supplemented with various metals (1 mM each metal separately) at 30°C for 32 h. Optical density at 600 nm (OD_{600nm}) was employed as the function of yeast growth.

Effect of heavy metals

The minimum inhibitory concentrations (MICs) were determined with bracketed concentrations of metals on YPD agar plates (Ilyas and Rehman 2015). The MIC was the metal concentration at which the isolates did not show any growth. The effect of heavy metals was determined by growing the yeast isolates in MSM broth in the presence and absence of heavy metals. Cultures were initially supplemented with 1 mM each of heavy metal and incubated at 30°C. Fresh weight (FW) was employed as the function of yeast growth in the presence of metal ions.

DNA extraction and PCR amplification

DNA was isolated and 18S ribosomal RNA (rRNA) genes were amplified by polymerase chain reaction (PCR) with oligonucleotide yeast primers, the forward primer (ITS-5; 5-GGAAGTAAAAGTCGTAACAACG-3) and the reverse primer (ITS-4; 5-TCCTCCGCTTATTGATATGC-3) (Larena et al. 1999). PCR was performed at 94°C for 4 min, annealing at 55°C for 2 min, and elongation at 72°C for 10 min. Amplified DNA was purified with Fermentas Gene Jet Gel Extraction kit. The PCR products were sequenced and analyzed at the Centre of Excellence in Molecular Biology, Thokar Niaz Baig, Lahore, Pakistan.

Protein estimation

Grown yeast cultures [24 h grown yeast cells at 30°C (pH 7)] were centrifuged at 1,400 x*g* for 10 min and phosphate buffer saline (PBS; pH 7) was used to wash the pellets twice and mixed in extraction buffer. Yeast cells were sonicated for 15 sec with 60 sec interval and centrifuged at 11,000 x*g* (4°C) for 10 min. The process of sonication was repeated 5 times for each sample. Bradford method (1976) was used to estimate protein contents of the samples by taking bovine serum albumin (BSA) as a standard. Extracts of protein were stored at -80°C for further use.

GSH, GSSG and NPSH determination

The protocol described by Anderson (1985) was used to estimate the total contents of GSH and non-protein thiol (NPSH) in cell lysates. Fresh, inoculated MSM broth medium was assigned as a control non-treated. Inoculated MSM broth (5×10^6 cells/ml), containing metal ions (1 mM) separately, was incubated at 30°C and at 120 rpm. Exponential-phase yeast cells were harvested by centrifugation (1,400 x*g* for 10 min) and washed twice with 1 mM phosphate-buffered saline (pH 7.0). Samples of biomass were suspended in 5% (w/v) sulfosalicyclic acid, sonicated for 15 s at 60 s intervals (5 cycles) and centrifuged (11,000 x*g* for 10 min) at 4°C. The GSH and GSSG contents were estimated by incubating the crude extract in buffer (100 mM phosphate buffer and 0.5 mM EDTA; pH 7.0) and 3 mM of 5-dithio-bis-(2 nitrobenzoic acid) at 30°C for 5 min. The reaction was started by adding 0.4 mM NADPH and 2 µl glutathione reductase. The reaction mixtures were kept at 30°C for 20 min before measuring the absorbance at 412 nm (Hitachi U-2800, Tokyo, Japan). The assay was calibrated with known concentrations of GSH and normalized to mM per gram of cells. Biomass prepared from oxidant free salt medium was taken as control. Cysteine and non-protein thiols were quantified by incubating reaction mixtures containing 100 µl of extracted sample (treated and untreated), 5 dithio-bis-(2-nitrobenzoic acid) (1 mM) and reaction buffer (0.1 M phosphate buffer and 0.5 mM EDTA) at 30°C for 10 min.

Biosorption

Yeast isolates were incubated in MSM medium at 30°C (pH 7) under shaking condition (120 rpm) and 5 ml aliquots were removed under sterilized conditions after time interval of 2, 4, 6, 8, 10, and 12 days. The samples were centrifuged at 4,000 *xg* for 10 min, collected culture pellets were weighed and washed with autoclaved distilled water afterwards divided into two parts. The first part was washed with 0.1 M EDTA for 10 min and the amount of metal associated with cell surface was removed as soluble fraction. The second part [(acid digested; 0.2 N HNO_3, H_2SO_4 (1:1)] was left on hot plate for 30 min. Each experiment was performed in thrice.

Total metal content present in the medium or processed by the yeast cells was estimated by flame atomic absorption spectrometer (Zeeman AAS, Z-5000 Model, Hitachi Ltd., Japan) and graphite furnace atomic absorption spectrometer (AA Solaar M6 Spectrometer, UK).

Polyacrylamide gel electrophoresis

Proteins were resolved by gel electrophoresis with 12% polyacrylamide gels. Metal-treated (1 mM each metal separately) and untreated cells were harvested [24 h grown yeast

cells at 30°C (pH 7)] and washed with phosphate buffer before sonication (5 cycles) at 4°C. After centrifugation (11,000 *xg* for 10 min), the supernatant fraction was precipitated with chilled 10% TCA and centrifuged at 11,000 *xg* for 15 min. Sample buffer was used for further solubilization of the cell extracts. The gels were run at 120 V as described by Laemmli (1970).

Statistical analyses

For each experiment, three independent measurements were taken and data shown are average values of means ± standard deviation (SD). Student's t-test was used to test significance between treatments and controls and SPSS version 15 was used for further analysis. Control group was treated identically without exposure to any treatment.

Results and Discussion

Growth conditions

Seven samples of industrial wastewater were collected and they ranged from 25°C to 38°C and pH 5 to 9. The 18S rDNA gene sequence (accession number KJ913820 and JN009854) of the isolates matched 96% similarity with *Trichosporon asahii* and 100% similarity with *Pichia kudriavzevii*. *T. asahii* grew best at 37°C and at pH 6, while *P. kudriavzevii* had best growth at 30°C (pH 7). In the presence of metals (100 mg/l), growth consistently slowed down (Fig. 1a, b). The order of growth inhibition in *T. asahii* was Pb>As>Cu>Cd and in *P. kudriavzevii* Pb>Cu>Cd>As. Biomass content (Table 1) of *T. asahii* and *P. kudriavzevii* also decreased in the medium containing metals (Table 1).

The tolerance to metals was analyzed by growing cells in medium supplemented with and without metals. The MIC values determined for *T. asahii* were 35 mM Pb, 33 mM Cu, 30 mM As, and 10 mM Cd while *P. kudriavzevii* showed resistance against heavy metal ions i.e. Pb (31 mM), Cu (27 mM), Cd (15 mM), and As (12 mM). Similar tolerances against As and Cd were also reported by *T. asahii* from the same laboratory (Ilyas et al. 2014).

Quantification of GSH NPSH and oxidative stress

The increased GSH levels are due to oxidative stress caused by test metals in the growth medium. GSH provides defense by mechanisms that involve binding of metals to complex formation, which is then removed by proteins that mediate vacuolar sequestration. Metal complexes react with (-SH) groups (protein glutathionylation), thus shielding them from irreversible metal binding, oxidative damage, and altering their oxidation states (Grant 2001, Khan et al. 2016).

T. asahii contained elevated GSH levels (83.1 µmol/g) in response to Cd in growth medium. Other metals, i.e. As (81.9 µmol/g), Pb (66.9), and Cu (56.2) also caused elevated GSH levels. The GSSG levels responded to As (22.4 µmol/g),

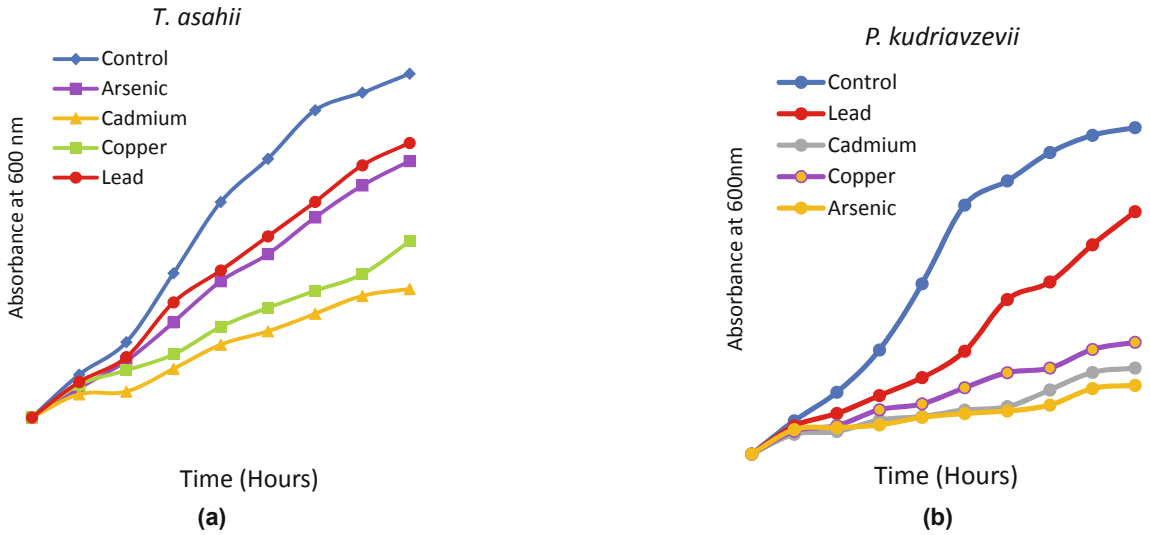

Fig. 1. Growth curves of *T. asahii* (a) and *P. kudriavzevii* (b) were obtained in MS broth supplemented with metal ions (1 mM each metal separately) at 30°C for 32 h. Optical density was taken at 600 nm

Table 1. Gram fresh weight (biomass) of environmental isolate *T. asahii* and *P. kudriavzevii* grown in MSM supplemented with and without heavy metals

Gram fresh weight expressed as% age		
Heavy metals used	***T. asahii***	***P. kudriavzevii***
Control (without heavy metal)	100	100
Cadmium Chloride ($CdCl_2$)	82 +/-2.9	78 +/-1.7
Sodium Arsenite ($NaAsO_2$)	87 +/- 0.2	65 +/- 2.5
Lead Nitrate $Pb(NO_3)_2$	85 +/-7.3	81 +/-0.13
Copper Sulfate ($CuSO_4$)	83 +/-11.5	79 +/-3.2

Pb (14.2) and Cu (19.3) but were unaffected by Cd (7.4). Control cells contained 7.0 µmol/g (Table 2). Enhanced GSH content in *P. kudriavzevii* was 52.8 µmol/g for Cd exposure and 56.5 for As, followed by 69.9 for Pb and 70.0 for Cu. GSSG levels were 14.8 for Cd, 7.0 for As, and 2.6 or Cu. Pb-treated cells and control cells contained 4.6 µmol/g and 3.0 µmol/g. In general, the presence of Cu and Pb the GSH/GSSG ratios were higher in *P. kudriavzevii* treated cells versus *T. asahii* (Fig. 2).

Metal exposure had resulted in higher intracellular pools of thiol compounds in the biomass and the accumulation changed the GSH/GSSG ratios. In the presence of Cu and Pbthe GSH/GSSG ratio was higher in *P. kudriavzevii* cells, indicating that GSH was acting as an antioxidant, which was in agreement with previous studies (Peña-Llopis et al. 2002). In comparison, the ratio decreased in As and Cd treated *P. kudriavzevii* and in all *T. asahii* metal treated cells except for Cd. The decreased GSH/GSSG ratio in As-treated *Saccharomyces cerevisiaeyap1* mutants has previously been reported by Menezes et al. (2008). Freeman et al. (1997) described the denaturation of protein, loss of protein function and thiol oxidation as consequences of GSH depletion under oxidative stress.

In *T. asahii,* the cysteine and NPSH contents were enhanced in response to contact with Cd (20%), As (13%) and Pb (5%), whereas Cu showed no response (Fig. 3). In *P. kudriavzevii* the NPSH levels were enhanced in all metal treatments (Fig. 3).

Metal binding proteins in the cytosol such as Cu/Cd binding proteins may also provide a good strategy to detoxify toxic metals. For example, cysteine and NPSH contents were strongly induced and enhanced by heavy metals (Ilyas et al. 2014, Khan et al. 2015a, b, 2016). Cysteine forms complexes with metal ions in the cytosol and subsequently transports them into the vacuole.

Heavy metal removal potential of yeasts

The uptake and removal of metals were determined over a 12-day exposure. *T. asahii* removed 78% with an uptake 44.8 mg Cd/g (Fig. 4a), whereas *P. kudriavzevii* removed 61% with an uptake 36.8 mg/g (Fig. 5a) from medium after 12 days

Table 1. Intracellular levels of reduced GSH, oxidized GSSG and total glutathione in *T. asahii* and *P. kudriavzevii* grown under optimum growth conditions [24 h grown yeast cells at 30°C (pH 7)] in MSM containing metal ions

Fungal Isolates	GSH (mM/g FW)	GSSG (mM/g FW)	GSH+GSSG (mM/g FW)
T. asahii without heavy metal	36.83+/-2.12	7.0+/-4.13	43.83+/-6.25
T. asahii with $CdCl_2$	83.06+/-0.08	7.40+/-3.87	90.46+/3.95
T. asahii with $NaAsO_2$	81.87+/-0.90	22.39+/-4.03	104.51+/-4.93
T. asahii with $Pb(NO_3)_2$	66.88+/-0.21	14.24+/-5.96	81.12+/-6.17
T. asahii with $CuSO_4$	56.19+/-0.64	19.35+/-3.37	75.69+/-4.01
P. kudriavzevii (without heavy metal)	29.80+/-0.25	3.09+/-2.84	32.9 +/-3.09
*P. kudriavzevii*with $CdCl_2$	52.76+/-.065	14.76+/-1.74	67.52+/-1.8
P. kudriavzevii with $NaAsO_2$	6.47+/-0.385	7.04+/-1.37	63.51+/-1.75
P. kudriavzevii with $Pb(NO_3)_2$	69.87+/-2.5	4.63+/-2.47	73.5+/-4.97
P. kudriavzevii with $CuSO_4$	69.9 +/-0.82	2.65+/-2.15	72.56+/-2.97

Experiments were performed in triplicate (n=3) and p<0.1where values were expressed as mean ± SE.

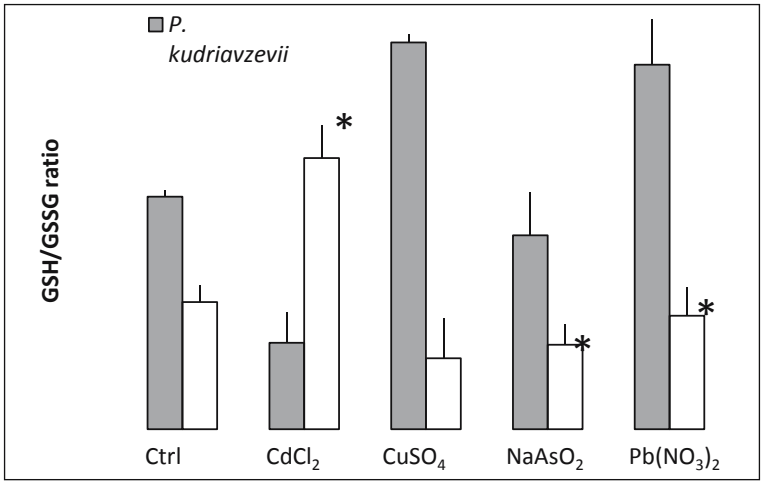

Fig. 2. GSH/GSSG ratio in T. asahii and P. kudriavzevii extracts cultured in the presence of Cd, As, Cu and Pb. Values were expressed as mean of ±SD where *p=0.05 and all experiments were performed in triplicate

of contact time. The removal of As by *T. asahii* was 72% and the uptake was 41 mg As/g whereas *P. kudriavzevii* removed 62% with an uptake 48 mg As/g after 12 days of contact time (Fig. 5b). Cu removal and uptake determined by *T. asahii* were 85% and 62 mg/g, while the uptake of 40 mg/g and 61% removal was established for *P. kudriavzevii*. Pb removal was 94.5 and 87% by *T. asahii* (Fig. 4d) and *P. kudriavzevii* (Fig. 5d) after 12 days of contact time.

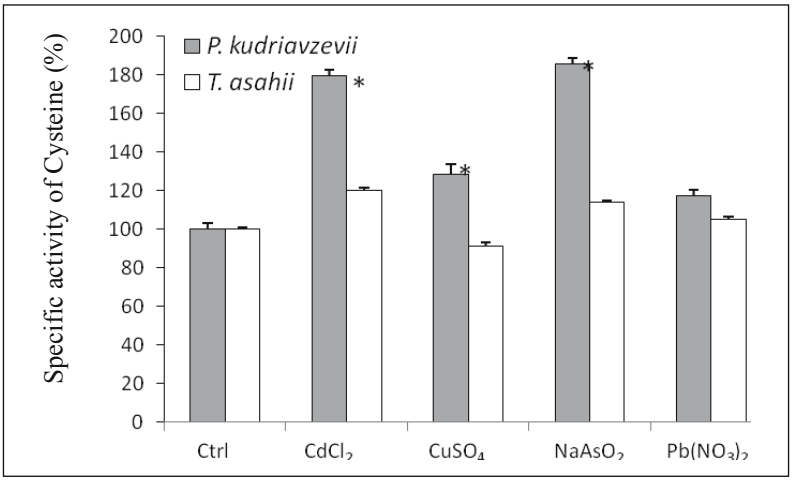

Fig. 3. Specific activity of cysteine (%) in *T. asahii* and *P. kudriavzevii* culturing in MSM broth medium with and without metal ions at 30°C and pH 7. Values were expressed as mean ± SD and all experiments were performed in triplicate where *p=0.05

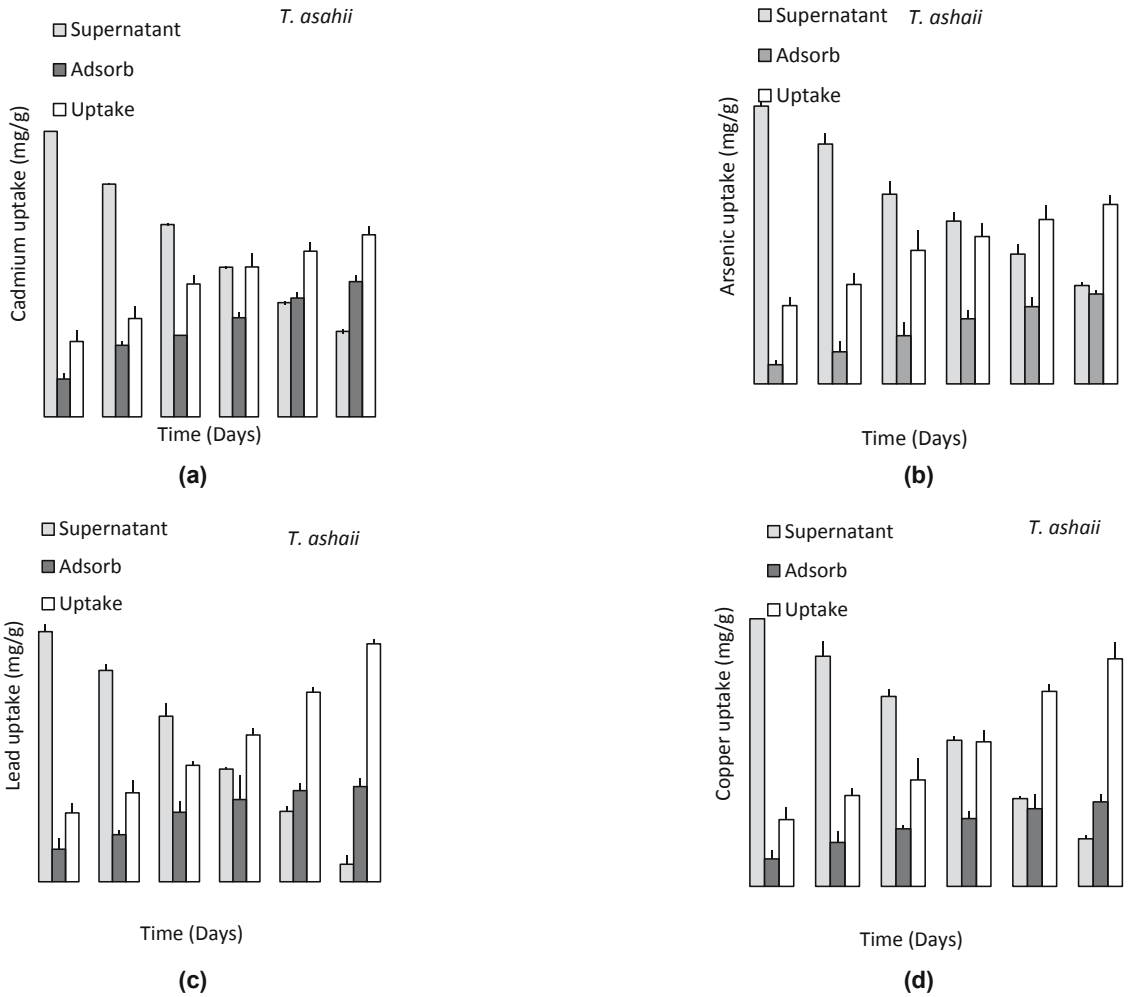

Fig. 4. Percentage (%) removal potential of cadmium (a) arsenic (b) lead (c) and copper (d) in *T. asahii*. Cultures were assayed for metal estimation by using atomic absorption spectrophotometer (AAS). Mean of three readings were plotted against time (days)

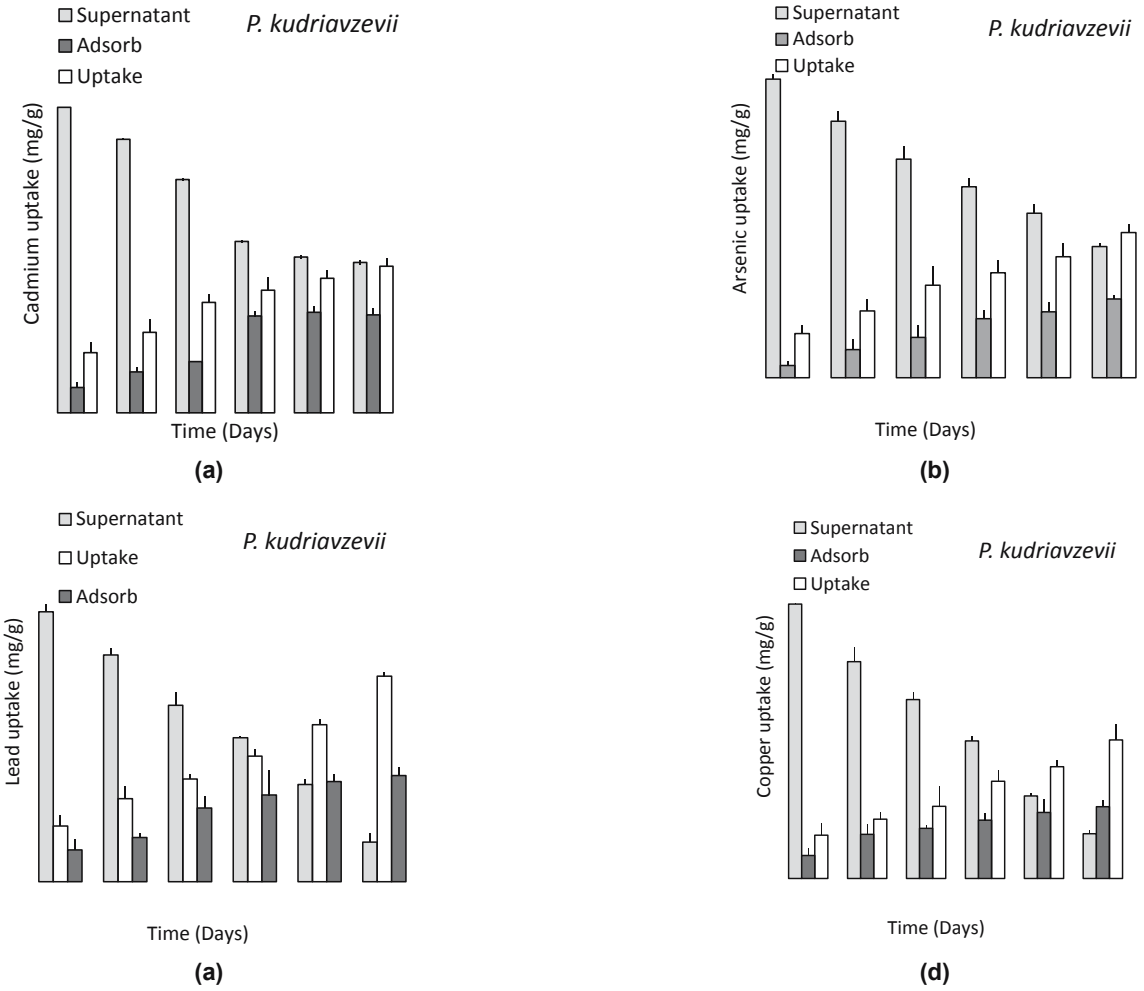

Fig. 5. *P. kudriavzevii* metal removal potential of cadmium (a) arsenic (b) lead (c) and copper (d). Cultures were assayed for metal estimation by using atomic absorption spectrophotometer (AAS). Mean of three readings were plotted against time (days)

The results suggest that *T. asahii* has multiple metal binding sites on the cell wall (Figs. 4, 5). In the case of *P. kudriavzevii*, Cd concentration increased in the medium after 8 days of contact time (Fig. 5a) whereas intracellular accumulation decreased. This trend suggested activation of an efflux system to export Cd from the cell.

Yeast cells have been extensively used in metal biosorption due to large cell size (0.0025–0.01 mm broad and 4.5–21 µm in length), easy cultivation, high yield of biomass, industrial safety to use and easy manipulation at morphological as well as genetic level (Shakya et al. 2015). Metal ions after adsorption on cell wall enter the yeast cell through channel proteins involved in the translocation of essential metal cations where they combine with GSH to form bis-glutathionato-metal (metal[GS]$_2$) complexes which are compartmentalized into vacuole by Ycf1 (Mielniczki-Pereira et al. 2008). Ycf1 is a vacuolar transmembrane transporter which belongs to ABC family and is directly involved in vacuolar accumulation of metal(GS)$_2$ complexes (Mazzola et al. 2015).

One dimensional gel electrophoresis

Differential protein expression was observed in metal-exposed cells. *T. asahii* cells treated with Cd and As revealed strong 80 and 20 kDa protein bands, which were much weaker for the Pb- and Cu-treated cells and the control (Fig. 6a). A weak 30 kDa protein band in metal-exposed samples was also observed when compared to control. Some protein bands of cysteine-rich MTs in the mass range <14 kDa were also found in metal-treated *T. asahii* and *P. kudriavzevii* samples (Fig. 6b).

Protein profiling by 1-DE revealed over-expression of MTs in response to heavy metals. The abundance of MTs in metal-treated yeast cells suggests a possible role in protection and survival of strains against ROS. Some protein bands that appeared in metal-treated samples were absent in non-metal treated samples. These proteins were of molecular weight and had intense bands in metals-treated yeast cells. Over-expression and disappearance of some protein bands in response to metal ions have been previously reported by Durve et al. (2013).

Conclusions

T. asahii and *P. kudriavzevii* were resistant to heavy metals. The MIC values varied and the growth of *T. asahii* and *P. kudriavzevii* was slower in metal treatments versus control. GSH and NPSH contents increased substantially and this up-regulation is believed to provide protection to the yeast from severe oxidative stress induced by metals. Increased oxidative stress confers increased resistance and adaptive response and may be critical for survival. Yeasts can accumulate heavy metals efficiently. 1DE analysis showed that some protein

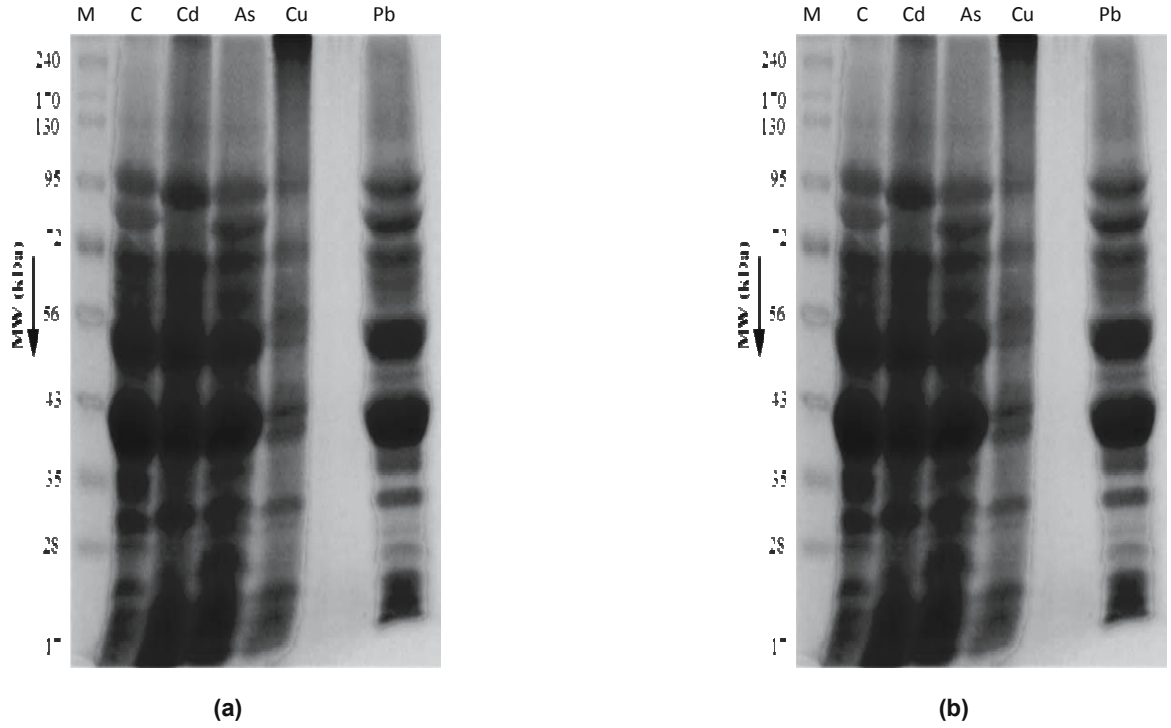

Fig. 6. Protein profiling of *T. asahii* (a) and *P. kudriavzevii* (b) cultivated in the presence and absence of heavy metals. Gels were stained with Coomassie brilliant blue R-250 for 40 minutes and intensity of the different proteins was noted. Lane M represents molecular mass protein makers

bands in metal-treated yeast cells were absent in the control and the production of low molecular weight MTs was abundant in metal-induced cells. High bioremediation potential makes these yeast strains suitable candidates to exterminate metal ions from the industrial wastewater. In future, after exploring their molecular biology they can become an attractive environmental tool for green chemistry.

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References

Anderson, M.E. (1985). Determination of glutathione and glutathione disulfide in biological samples, *Methods in Enzymology,* 113, pp. 548–555.

Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein--dye binding, *Analytical Biochemistry*, 7, pp. 248–254.

Celebi, N., Nadaroglu, H., Kalkan, E. & Kotan, R. (2016). Removal of copper from copper-contaminated river water and aqueous solutions using *Methylobacterium extorquens* modified Erzurum clayey soil, *Archives of Environmental Protection*, 42, pp. 58–69.

Cotgreave, I.A. & Gerdes, R.G. (1998). Recent trends in glutathione biochemistry-glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation?, *Biochemical and Biophysical Research Communications*, 242, pp. 1–9.

Dickinson, D.A. & Forman, H.J. (2002). Glutathione in defense and signaling, *Annals of the New York Academy of Sciences*, 973, pp. 488–504.

Durve, A., Naphade, S., Bhot, M., Varghese, J. & Chandra, N. (2013). Plasmid curing and protein profiling of heavy metal tolerating bacterial isolates, *Archives of Applied Science Research*, 5, pp. 46–54.

Febrianto, J., Kosasih, A.N., Sunarso, J., Ju, Y.-H., Indraswati, N. & Ismadji, S. (2009). Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies, *Journal of Hazardous Materials*,162, pp. 616–645.

Freeman, M.L., Huntley, S.A., Meredith, M.J., Senister, G.A. & Lepock, J. (1997). Destabilization and denaturation of cellular protein by glutathione depletion, *Cell Stress and Chaperones*, 2, pp. 191–198.

Grant, C.M. (2001). Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions, *Molecular Microbiology*, 39, pp. 533–541.

He, M., Li, X., Liu, H., Miller, S.J., Wang, G. & Rensing, C. (2011). Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain Lysinibacillus fusiformis ZC1, *Journal of Hazardous Materials*, 185, pp. 682–688.

Huang, F., Guo, C.-L., Lu, G.-N., Yi, X.-Y., Zhu, L.-D. & Dang, Z. (2014). Bioaccumulation characterization of cadmium by growing Bacillus cereus RC-1 and its mechanism, *Chemosphere*, 109, pp. 134–142.

Ilyas, S. & Rehman, A. (2015). Oxidative stress, glutathione level and antioxidant response to heavy metals in multi-resistant pathogen, Candida tropicalis, *Environmental Monitoring and Assessment*, 187(1), pp. 1–7.

Ilyas, S., Rehman, A., Varela, A.C. & Sheehan, D. (2014). Redox proteomics changes in the fungal pathogen Trichosporon asahii on arsenic exposure: Identification of protein responses to metal-induced oxidative stress in an environmentally-sampled isolate, *PloS One*, 9(7), e102340.

Khan, Z., Hussain, S.Z., Rehman, A., Zulfiqar, S. & Shakoori, A.R. (2015a). Evaluation of cadmium resistant bacterium, Klebsiella pneumoniae, isolated from industrial wastewater for its potential use to bioremediate environmental cadmium, *Pakistan Journal of Zoology*, 47, pp. 1533–1543.

Khan, Z., Nisar, M.A., Hussain, S.Z., Arshad, M.N. & Rehman, A. (2015b). Cadmium resistance mechanism in Escherichia coli P4 and its potential use to bioremediate environmental cadmium, *Applied Microbiology and Biotechnology*, 99, pp. 10745–10757.

Khan, Z., Rehman, A. & Hussain, S.Z. (2016). Resistance and uptake of cadmium by yeast, Pichia hampshirensis 4Aer, isolated from industrial effluent and its potential use in decontamination of wastewater, *Chemosphere*, 159, pp. 32–43.

Kim, H.S., Kwack, S.J. & Lee, B.M. (2005). Alteration of cytochrome P-450 and glutathione S-transferase activity in normal and malignant human stomach, *Journal of Toxicology and Environmental Health*, 68, pp. 1611–1620.

Klatt, P. & Lamas, S. (2000). Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress, *European Journal of Biochemistry*, 267, pp. 4928–4944.

Laemmli, U.K. (1970). Cleavage of structural proteins during assembly of the head of the bacteriophage T4, *Nature*, 227, pp. 680–685.

Larena, I., Salazar, O., Goncalez, V., Julian, M.C. & Rubio, V. (1999). Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes, *Journal of Bacteriology*, 75, pp. 187.

Mazzola, D., Pimentel, C., Caetano, S., Amaral, C., Menezes, R., Santos, C.N., Eleutherio, E. & Rodrigues-Pousada, C. (2015). Inhibition of Yap2 activity by MAPKAP kinase Rck1 affects yeast tolerance to cadmium, *FEBS letters,* 589, pp. 2841–2849.

Menezes, R.A., Amaral, C., Batista-Nascimento, L., Santos, C., Ferreira, R.B., Devaux, F., Eleutherio, E.C. & Rodrigues-Pousada, C. (2008). Contribution of Yap1 towards Saccharomyces cerevisiae adaptation to arsenic-mediated oxidative stress, *Biochemistry Journal*, 414, pp. 301–311.

Mielniczki-Pereira, A.A., Schuch, A.Z., Bonatto, D., Cavalcante, C.F., Vaitsman, D.S., Riger, C.J., Eleutherio, E.C.A. & Henriques, J.A.P. (2008). The role of the yeast ATP-binding cassette Ycf1p in glutathione and cadmium ion homeostasis during respiratory metabolism, *Toxicology Letter,* 180, pp. 21–27.

Pe˜na-Llopis, S., Ferrando, M.D. & Pe˜na, J.B. (2002). Impaired glutathione redox status is associated with decreased survival in two organophosphate poisoned marine bivalves, *Chemosphere*, 47, pp. 485–497.

Penninckx, M.J. (2002). An overview on glutathione in Saccharomyces versus non-conventional yeasts, *FEMS Yeast Research*, 2(3), pp. 295–305.

Shakya, M., Sharma, P., Meryem, S.S., Mahmood, Q. & Kumar, A. (2015). Heavy metal removal from industrial wastewater using fungi: Uptake mechanism and biochemical aspects, *Journal of Environmental Engineering,* C6015001.

Uzun, Y. & Sahan, T. (2017). Optimization with Response Surface Methodology of biosorption conditions of Hg(II) ions from aqueous media by Polyporus Squamosus fungi as a new biosorbent, *Archives of Environmental Protection*, 43, pp. 37–43.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease, *International Journal of Biochemistry and Cellular Biology*, 39, pp. 44–84.

Vargas-García, M.dC., López, M.J., Suárez-Estrella, F. & Moreno, J. (2012). Compost as a source of microbial isolates for the bioremediation of heavy metals: In vitro selection, *The Science of Total Environment*, 431, pp. 62–67.

Yang, T., Chen, M.-L. & Wang, J.-H. (2015). Genetic and chemical modification of cells for selective separation and analysis of heavy metals of biological or environmental significance, *Trends in Analytical Chemistry*, 66, pp. 90–102.

Yan, G. & Viraraghavan, T. (2003). Heavy-metal removal from aqueous solution by fungus Mucor rouxii, *Water Research*, 37(18), pp. 4486–4496.