Global Journal of Pharmacology 8 (3): 336-339, 2014
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2014.8.3.82345

Proteomic Profile in Heavy Metal Treated *Saccharomyces cerevisiae* of Baker's Yeast

Manoj Adhikari, Asal Mohseni Sajadi Eraghi, Prasant Rout,
Radhakrishnan Senthilkumar and Selvam Arjunan

Indian academy Degree College, Hennur cross, Bangalore 560043, India

Abstract: The presence of yeast strains that are resistant to the heavy metal ions in industrial effluents shows the capability of these strains to resist stressful condition. However, heavy metals at extreme concentrations potentially have greater impact on microbial community structure, biomass and activities. The undesirable effect of heavy metals has often been observed as a reduction in microbial biomass and activity. As the presence of more metals could become an intense challenge to the maintenance of a phylogenetically and functionally different microbial community; resistance mechanisms may not offer protection and it might be toxic for cells. In order to assess the characteristic toxic effect exerted by the heavy metals like chromium and lead on the yeast cells, changes in protein level was studied. Using the MALDI TOF, the trypsinized protein from *S. cerevisiae* treated with chromium and lead were analyzed for the mass to charge ratio. This analysis identified a total of 37 tryptic peptides with a mass: charge ratio (m/z) between 600.307 and 2,968.59 Da, there are some common proteins as well as some unique proteins were identified independently of chromium and lead treated. *Saccharomyces cerevisiae* is identified and the peptide of Tropomyosin and Isoamyl acetate-hydrolyzing esterase for chromium treated *S. cerevisiae* have been identified. While for the lead treated *S. cerevisiae*, the peptide of ORF 2310 and Cylin-5 have been identified by using MASCOT software. These results can give an insight into the mechanism of the toxic effect as well as genotoxicity put forth by heavy metals comprising of lead and chromium treated.

Key words: *Saccharomyces cerevisiae* · Maldi Tof · Chromium · Lead · Proteome

INTRODUCTION

A major concern in many of the countries is the heavy metal pollution owing to their presence in drinking water and wastewater usually in an exceeding level than the acceptable quantity. The toxic effects of the these heavy metals, which are found particularly in animals of higher tropic levels including humans, are considered to be due to the presence of the metal ions in the environment that are bio-magnified and accumulated in the food chain and tissues, respectively.

The metals, which are hazardous to humans, comprise of lead, cadmium, mercury, arsenic, copper, zinc and chromium. Among these metals, mercury is known to cause mutation and genetic damage. Copper, lead and chromium can cause injury to the brain and bone [1]. These heavy metals that are most commonly derived from the industries like electroplating and battery factories, metal finishing and chemical manufacturing can be hazardous to the human health and ecosystem [2].

Different techniques, which are employed to remove the heavy metals from the waste water, include ion exchange, evaporation, precipitation, membrane separation. The disadvantages of these conventional techniques are high cost, incomplete metal recovery, high energy requirement and generation of toxic sludge which require disposal. Consequently, an alternative technique called biosorption, which involves the removal of heavy metals by inert binding to the biomass from aqueous solution, has emerged with more advantages over the conventional method [3]. The process of biosorption is appealing because of the exploitation of the naturally occurring biomass or the biomass which has been used for the various industrial processes including fermentation. The other advantages include low operating cost,

Corresponding Author: Selvam Arjunan, Department of Biotechnology Indian academy Degree College Hennur cross, Hennur Main Road Kalyan Nagar, Bangalore 560043, India.
Tel: +91-7760867365.

effective in dilute solutions, produce lowest secondary waste, involve less time to complete the process and have no toxicity limit for heavy metals. Thus, the biosorbents, which have the capacity to seize heavy metals, are yeast, bacteria, algae and fungi [4].

With the availability of huge quantity of spent fungal biomass from the fermentation industries and its compatibility to structural and functional changes, fungal biosorption have been studied extensively [5]. Among the various fungi including Rhizopus, Aspergillus, Streptoverticillum, Phanerochaete and Saccharomyces, which have the biosorption potential, *Saccharomyces cerevisiae* have been demonstrated to be the efficient biosorber of heavy metals like Au, Mn, Cu, Co, Pb [6-8]. In the revelation of the above findings, the objective of the present study is to evaluate the characteristic toxic effect exerted by chromium and lead on *Saccharomyces cerevisiae* with the changes in protein level measured by mass spectrometry.

MATERIALS AND METHODS

Materials: Bakery Yeast samples were collected from market. Chromium and Lead were got it from department of chemistry, Indian academy degree college, Bangalore-43, Atomic absorbance spectrophotometer (AAS) in Department of soil science and agricultural chemistry, College of agriculture, GKVK, Bangalore-65. Mass Spectrometry facility was used in Indian Institute of Science (IISc), Bangalore, India. YEPD agar was purchased from Vasa Scientific Pvt Ltd.

Methods

Inoculation and Isolation Yeast: YEPD agar plates were prepared and bakery yeast was inoculated. After inoculation the petri plates were incubated at 30°C for up to 8 days to check the appearance of yeast colonies. Colonies appearing on these plates were examined under low magnification for their morphology, which facilitated the selection of different colonial forms. Selected, single, well-isolated colonies brought into culture by re-plating on YEPD agar medium.

Protein Estimation: In order to extract the protein from the yeast, cells were homogenized with liquid nitrogen using mortar pestle and added 20il of protein extraction buffer (50 mM Tris-HCl, 2% SDS, 10mM DTT, 10% Glycerol). Homogenized mixture was centrifuged at 10,000 rpm for 5 min and proteins were precipitated with ice-cold acetone. The protein mixture was dissolved in 2% SDS solution and storied at -20°C. The Proteins concentration was estimated by Bradford assay.

Mass Spectrometry Analysis: Proteolysis was performed overnight at 37° C with Trypsin. After cleavage, the peptide mixture was centrifuged, dried and dissolved for mass spectrometric analysis. Centrifuged sample were placed on MALDI plate followed by 0.5 µL of alpha-cyano-4-hydroxycinnamic acid matrix (10mg/ml in 50% acetonitrile, 0.1% TFA). Allowed the spots to dry completely and loaded the plate into Voyager. Proteins were identified with peptide mass fingerprinting data using Mascot (http://www.matrix science.com). Mascot Distiller was used to detect peaks to fit an ideal isotopic distribution to the experimental data.

RESULTS

The soil sample and Baker's yeast sample were serial diluted and grown on YEPD media and isolated pure colony of *S. cerevisiae* after several times of selection and subculture on YEPD agar medium.

As figure 1 showed that the numbers of colony of yeast were maximum at 100 µg/ml of lead and chromium enrichment YEPD agar media. The growth rate of yeast on Lead containing media is maximum rather than Chromium contain YEPD agar medium. This result reflects that Chromium is more toxic to yeast than Lead. Even though the yeast is resistance to the Chromium and Lead and higher concentration of heavy metal is leading programme cell death.

Biosorption: Biosorption of heavy metal in yeast is defining as the amount of heavy metal taken by the yeast cell during the treatment with heavy metal using atomic absorbance spectrophotometer (AAS). In order to assay the biosorption, the chromium treated yeast extract solution was measured at 357.9 nm, where as Lead treated was measured at 217.0 nm. The result is shown below table and bar graph representation.

The reliable and correct measurement of biosorption of heavy metal is done by Atomic absorption Spectrophotometer in different wavelength depending upon the type of heavy metal. The Biosorption of heavy metal by yeast is increasing order with respect to higher concentration of heavy metal. Yeast has absorbed maximum 170.28 ppm Lead when yeast is grown in 500µg/ml concentration of Lead containing YEPD broth

Table 1: Biosorption of Heavy Metal (Pb and Cr) By Bakery Yeast measured by AAS

	Parameters			
	Chromium (Cr) @ 357.9nm (ppm)		Lead (Pb) @ 217.0 nm (ppm)	
Yeast extract Liquid Samples	Initial (0 Day)	Final (8 Day)	Initial (0 Day)	Final (8 Day)
10µg/ml	3.85	3.55	4.331	3.36
50µg/ml	19.26	18.18	21.65	5.82
100µg/ml	38.53	34.75	43.31	5.94
500µg/ml	192.65	53.53	216.55	46.27

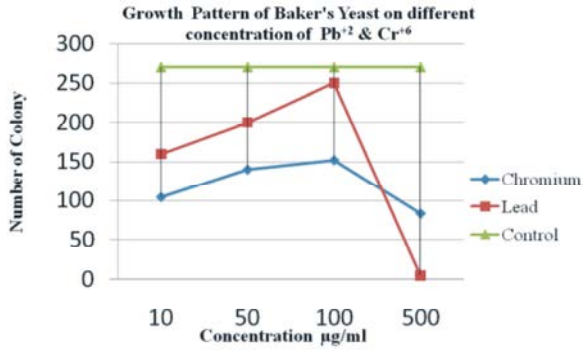

Fig. 1: Inhibitory concentration of heavy metal on S. *cerevisiae (Baker's* Yeast)

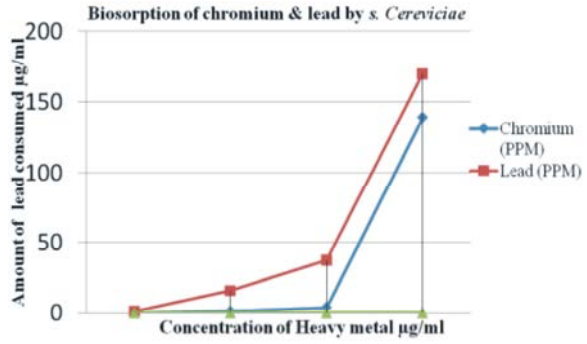

Fig. 3: Biosorption of Heavy Metal (Pb and Cr) by bakery yeast

medium where as minimum 0.3086 ppm absorbed after treatment of 10µg/ml lead. In case of Chromium treated yeast, maximum 139.12 ppm of Chromium was absorbed by yeast after treatment of 500 µg/ml chromium to yeast.

Mass Spectrometry Analysis: It has also been found that *S. cerevisiae* treated with heavy metal at a concentration of 100µg/ml expressed more proteins. While at 500µg/ml, the protein expressed by *S. cerevisiae* was lesser. Using the MALDI-TOF, the trypsinized peptides were from *S. Cerevisiae* treated with chromium and lead was analyzed by mass to charge ratio. As the result, totally 37 tryptic peptides with a mass: charge ratio (m/z) between 600.307 and 2,968.59 Da. *Saccharomyces cerevisiae* is identified and the peptides of Tropomyosin and Isoamyl acetate-hydrolyzing esterase for chromium treated *S. Cerevisiae* have been uniquely identified. Similarly, while for the lead treated *S. Cerevisiae*, the peptide of ORF 2310 and Cylin-5 have been identified by using MASCOT software.

DISCUSSION

It has been found that in waters receiving industrial effluents, a large variety of microorganisms including yeast are present. Only strains which can resist heavy metal ions are able to survive in the industrial effluents rich in metal ions. Thereby, they have developed strategies to resist, tolerate, metabolize and to detoxify these toxic levels of heavy metals for their survival. The presence of yeast strains that are resistant to the heavy metal ions in industrial effluents shows the capability of these strains to resist stressful condition. However, heavy metals at extreme concentrations potentially have greater impact on microbial community structure, biomass and activities. The undesirable effect of heavy metals has often been observed as a reduction in microbial biomass and activity [9].

Since the presence of more metals could become as an intense challenge to the maintenance of a phylogenetically and functionally diverse microbial community; resistance mechanisms may not offer protection and it might be toxic for cells [10]. In the present study we analyzed the tolerance levels of heavy metals on baker's yeast which is identified as *Saccharomyces cerevisiae* species. It was found that the minimum inhibitory concentration of heavy metal including chromium and lead in the YEPD agar media against the growth of yeast was >100 µg/ml. This indicates that the high amount of Chromium and Lead is toxic to *Saccharomyces cerevisiae.*

Using atomic absorption spectrophotometer, a reliable and correct measurement of biosorption of heavy metal is measured in different wavelength based on the type of heavy metal used. Yeast has absorbed

maximum 170.28 ppm Lead when yeast is grown in 500µg/ml concentration of Lead containing YEPD broth medium where as minimum 0.3086 ppm absorbed after treatment of 10µg/ml lead. In case of Chromium treated yeast, maximum 139.12 ppm of Chromium was absorbed by yeast after treatment of 500 µg/ml chromium to yeast. Thus, the biosorption of both the heavy metal was higher at an increased concentration of 500µg/ml [10].

CONCLUSION

The biosorption of lead and chromium by *Saccharomyces cerevisiae* resulted in different unique protein expression profile, using MALDI-TOF [11], *Saccharomyces cerevisiae* species were reliably identified and also, the chromium and lead exposed yeast proteome is also analysed. However, future studies must delineated details of the signaling pathway and adaptation of heavy metal treated *Saccharomyces cerevisiae.*

ACKNOWLEDGEMENT

I would like to thank Prof C. Chennarayappa, HOD, Department of Chemistry, Indian Academy Degree College and Bangalore-43 for help and support.

REFERENCES

1. Cerulli, J., D.W. Grabe, I. Gauthier, M. Malone and M.D. McGoldrick, 1998. Chromium picolinate toxicity. Ann Pharmacother, 32(4): 428-31.
2. Apiratikul, R., T.F. Marhaba, S. Wattanachira and P. Pavasant, 2004. Biosorption of binary mixtures of heavy metals by green macro alga, Caulerpa lentillifera. Songklanakarin J. Sci. Technol., 26(Suppl. 1): 199-207.
3. Vijayaraghavan, K. and Y.S. Yun, 2008. Biosorption of C.I. Reactive Black 5 from aqueous solution using acid-treated biomass of brown seaweed Laminaria sp. Dyes Pigm., 76: 726-32.
4. Rajesh Dhankhar Anju, 2011. Hooda Radha Solanki Poonam Ahlawat Sainger *Saccharomyces cerevisiae*: a potential biosorbent for biosorption of uranium. International Journal of Engineering Science and Technology (IJEST)., 3(6): 5397-5401.
5. Lin, Z., J. Wu, R. Xue and Y. Yang, 2005. Spectroscopic characterization of Au3+ biosorption by waste biomass of *Saccharomyces cerevisae*. Spectrochim. Acta. Microbiol., 40: 535-9.
6. Parvathi, K., R. Nareshkumar and R. Nagendran, 2007. Biosorption of manganese by A. niger and S. cerevisiae. World J. Microbiol. Biotechnol., 23: 671-676.
7. Mishra, A., N. Pradhan, R.N. Kar, L.B. Sukla and B.K. Mishra, 2009. Microbial recovery of uranium using native fungal strains, Hydrometallurgy, 95: 175-177.
8. Pakshirajan, Kannan, Swaminathan and Thiyagarajan, 2010. Biosorption of lead by the immobilised fungus Phanerochaete chrysosporium in a packed bed column. International Journal of Environmental Technology and Management, 12: 214-228.
9. Perez, R.R., C.A. Sousa, T. Vankeersbilck, M.D. Machado and E.V. Soares, 2013. Evaluation of the role of glutathione in the lead-induced toxicity in Saccharomyces cerevisiae. Curr Microbiol., 67(3): 300-5.
10. Kordialik-Bogacka, E. and A. Diowksz, 2014. Metal uptake capacity of modified Saccharomyces pastorianus biomass from different types of solution. Environ Sci. Pollut Res. Int., 21(3): 2223-9.
11. Pinto, A., C. Halliday, M. Zahra, S. van Hal, T. Olma, K. Maszewska, J.R. Iredell, W. Meyer and S.C. Chen, 2011. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Identification of Yeasts Is Contingent on Robust Reference Spectra. PLoS ONE., 6(10): e25712.