A STUDY ON ANTIOXIDANT ACTIVITY AND MEMBRANE INTEGRITY OF *CANDIDA* SP. STRAIN Y-01 UPON PB STRESS

Bhavya G*., Abhijith P., Ravikant S., and Geetha N[1].
Eco-Biotech Laboratory, DOS in Biotechnology, University of Mysore,
Manasagangotri, Mysuru-06, Karnataka, India
[1]geethabiotech.uom@gmail.com

Abstract: In nature, the microorganism's, especially fungi are developed with a different metabolic strategy' to combat the adverse effects of metal-induced toxicity thereby decreasing their scathe. In the current work, the *Candida* sp. strain Y-01 and SIE - 01 have shown resistance to Pb^{2+} in the concentration range of 0.2mM to 2.0mM. Further exploring the distinguishable cause of resistance, the antioxidant defence enzyme system and the membrane integrity of cells were analyzed with respect to 0.8mM concentration of Pb^{2+} at 72hr of incubation. On evaluating the CAT and MDA contents, the increased CAT and decreased MDA activities was observed in *Candida* sp. strain Y-01 in comparison with the control. The increased CAT indicates the possible key role of CAT related pathway for the resistance mechanism. The decreased MDA indicated the lower oxidative stress to membrane component, which was justified as membrane integrity of cell found to be around 85% upon stress with respect to the control. The increased CAT, MDA and decreased membrane integrity (45%) were observed in SIE – 01. The increased MDA indicated the oxidative lipid peroxidation of cell membrane component thus leading to loss of cell membrane integrity, changes in membrane fluidity and metabolic process in the yeast isolate SIE -01. The results suggest that the application of *Candida* sp. strain Y-01 for bioremediation of Pb^{2+} contaminated site and use of CAT and MDA as biomarkers for metal-induced stress.

Key words – *Candida* sp., CAT, MDA, Pb^{2+}

I. Introduction

Heavy metals and various metalloids are an integral part of our environment and are widespread in nature. Anthropogenic activities have enhanced the re-distribution of many toxic heavy metals from the earth's crust to different environmental compartments as a result of the industrial revolution (Gadd & Griffiths, 1977). Heavy metal act as toxic pollutants worldwide posing a rising threat to both environment and human health. Heavy metals cause various damaging effects in plants by inducing various morphological, physiological, and biochemical dysfunctions, either directly or indirectly. Heavy metals (e.g, Pb, Cd, Ni, AI, Mn and Zn) cannot generate ROS directly by participating in biological redox reactions such as Haber Weiss/Fenton reactions, which is unlike to redox-active metals such as iron and copper.

The overproduction of Reactive oxygen species (ROS) is the earliest consequence of heavy metal toxicity in a plant or microbial cell like yeasts. These heavy metals induce ROS generation via different indirect mechanisms, such as stimulating the activity of NADPH oxidases, displacing essential cations from specific binding sites of enzymes etc., Enhanced generation of ROS species from heavy metal toxicity deteriorates the intrinsic antioxidant defense system of yeast cells, and causes oxidative stress (Mesquita *et al.,* 2016). As a result of the interaction between ROS and biomolecules, yeast cells under oxidative stress display various chemical, biological and physiological toxic symptoms.

Heavy-metal-induced ROS in yeast cells cause lipid peroxidation, membrane dismantling and damage to DNA, protein and carbohydrates. Yeasts like plants have very well-organized defense systems, consisting of enzymatic and non-enzymatic antioxidation processes (Ilyas and Rehman, 2015). The primary defense mechanism for heavy metal detoxification is the reduced absorption of these metals into plants or their sequestration in root cells. Secondary heavy metal tolerance mechanisms in yeasts include activation of antioxidant enzymes and the binding of heavy metals by amino acids, glutathione and phytochelatins (Ilyas *et al.,* 2016). These defense systems work in combination to manage the cascades of oxidative stress and to defend yeast cells from the toxic effects of ROS.

In the present study we have focused on the biochemical processes involved in the over production of ROS in the *Candida* sp. strain Y-01 upon Pb stress as an aftermath to heavy metal exposure. We have also described the ROS scavenging process that is associated with the antioxidant defense machinery in this fungal species. Eventually, the mechanism of membrane integrity on the biochemical basis of heavy metal detoxification in *Candida* sp was elucuidated by using biotechnological tools.

II. Materials and Methods

2.1 Pure culture maintenance

The isolated yeast cultures - *Candida* sp. strain Y-01 and SIE – 01 from contaminated sites were sub-cultured on yeast potato dextrose agar media (YPDA) and maintained at room temperature.

2.2 Preparation of Pb^{2+} stock solution

100ml of 50mM concentration of Lead nitrate ($Pb(NO_3)_2$) solution was prepared in distilled water and stored at room temperature for further use.

2.3 Determination of metal tolerance and growth curve analysis

Log phase cultures of *Candida* sp. strain Y-01 and SIE – 01 were incuolated separately into YPD broth amended with 0 to 1.6mM Pb^{2+}. Cultures were incubated at room temperature for 5days. For every 24hr of interval the optical density was measured at 600nm and recorded. The culture media without metal amendement was taken as control. A graph was ploted against time interval at different concentration (Fig.1 and 2).

2.4 Estimation of antioxidant assays

2.4.1 Protein extraction and determination of total protein

The cells were grown for 72hr in YPD broth as control and also amended with 0.8mM Pb in YPD broth. The cells were pelleted by centrifugation at 10,000 rpm for 10min. Cells were homogenized in 50mM potassium phosphate buffer, centrifuged at 5000 rpm at 4^0C for 10 min. The supernant was stored at 4^0C, until further analysis.The total protein content in the cell free extract was deteremined by Lowry's method using BSA as standard with modification (Lowry *et al*., 1951).

2.4.2 Enzyme activities

2.4.2.1 Catalase (CAT)

The catalase (CAT) activity was determined according to Zeng *et al*. (2012) with slight modification. The 1ml of assay solution contained 10µl of crude enzyme, 990µl phosphate buffer containing 10mM H_2O_2. The decrease in the absorbance was monitored at 240nm for two minutes using spectrophotometer. The activity was expressed as µmoles of H_2O_2 decomposed/min/mg of protein (Fig. 3).

2.4.2.2 Malondialdheyde (MDA) estimation by MDA-TBARS assay

MDA assay and calculation was carried out according to Wang *et al.* (2014) with modification. The reaction mixture contained the equal volume of enzyme extract and 0.8% thiobarbituric acid in 15% TCA. This was incubated at 95^0C for 40min and cooled on ice. The MDA-TBA content was centrifuged at 6000rpm for 10min at 4^0C. The absorbance of supernatant was measured at 456, 532 and 600nm. The MDA content was calculated using the formula MDA (µM/g) = $6.45(A_{532}-A_{600})-0.56(A_{456})$ and expressed as µM/g (Fig. 4).

2.5. Membrane Integrity test

The test was followed according to Kwolek-Mirek and Zadrag-Tecza (2014) with slight modification. The isolates were grown in YPD broth with and without 0.8mM Pb stress. The cells were pelleted and suspended in PBS. The concentration was maintained at 1 x 10^6cells/ml. The 10µl cell suspension was treated with 10µl 0.1%

trypan blue along with 980 µl of PBS. The number of viable and non-viable cells were counted by visualizing under microscope. The unstained cells were counted as membrane integrated cells and stained as membrane disturbed cells. The percentage of membrane integrity was calculated (Fig. 5).

III. Result and Discussion

Heavy metal toxicity continues to be a major problem at several levels, and there is a pressing need for the deleterious effects of metals at the cellular and molecular level. Microbial systems like yeasts have developed with a strong dependence on transition metals for accomplishing a number of biochemical reactions. Iron, copper, manganese and zinc are essential for virtually all forms of life with their unique chemistries contributing to a variety of physiological processes including oxygen transport, generation of cellular energy and protein structure and function. Properties of these metals that make them so essential to life also make them extremely cytotoxic in many cases through the formation of damaging oxygen radicals (Hosiner *et al*., 2014).

The extent of the contribution to whole-cell metal toxicity made by damage to each potential target has not been determined, and this is not helped by the fact that almost all studies have tended to focus on one type of target and/or whole cells exclusively. The evidence to date indicates that each of the major cellular macromolecules can be a target for metal toxicity. The relative importance of membrane damage in determining the metal sensitivities of different yeasts may depend on the organism's lipid compositions, specifically their polyunsaturated fatty acid contents (Avery, 2001).

The growth of the *Candida* species was seen under all concentration of metal with least at 1.6mM. At 0.4mM showed the prolonged log phase compare to control, whereas in 1.6mM showed prolonged lag phase (Fig. 1). The growth of the SIE-01 was found to be more vigorous in the presence of Pb than in the control, with a prolonged lag as well as log phase. The prolonged lag and log phases might indicate the time required for adapatability by the isolates to grow under stress conditions.

The Catalase activity (CAT) in the tested isolates was found to increased by 50% under Pb stress, indicating the role of CAT in scavenging the ROS and decompositing. The lipid peroxidation in the cell is marked by the MDA content. The slight increase in MDA in comparison to control was observed in SIE -01, whereas in *Candida* sp it was found to be decreased. The loss of membrane integrity of fungal cells was seen in higher percentage in the SIE -01 whereas in *Candida* sp. Strain Y-01 its almost negligiable in comparison with the control.

While life has evolved to exploit the chemistries of transition metals to drive physiological reactions, systems have concomitantly evolved to protect against the damaging effects of these same metals. Yeasts like *Saccharomyces cerevisiae* and *Candida* sps are valuable tools for studying metal homeostasis with many of the genes identified thus far having homologs in higher eukaryotes including humans.

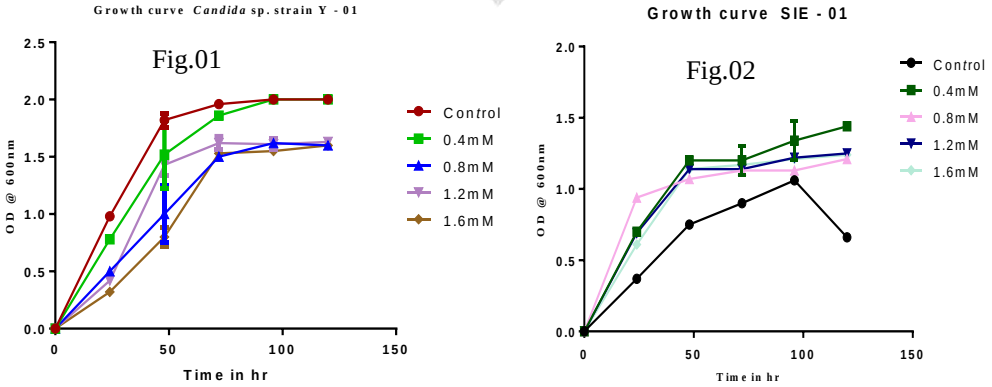

Fig. 1: The growth pattern of *Candida* sp. strain Y-01 under different Pb concentration.

Fig.2: The growth pattern of SIE - 01 under different Pb concentration.

Fig 3

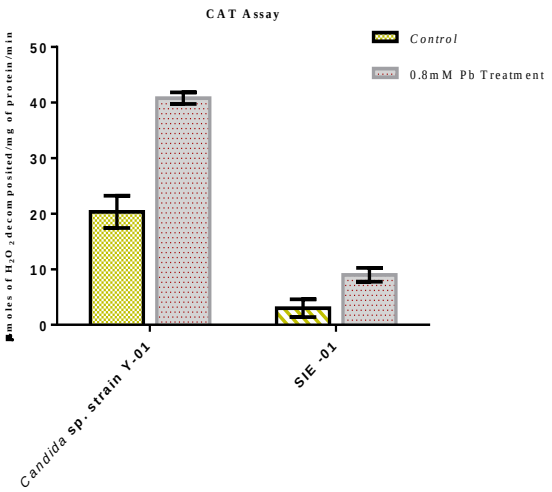

Fig 4

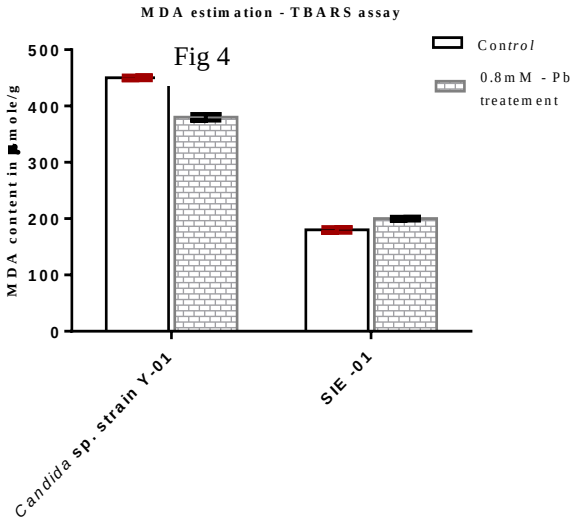

Fig. 3: Catalase activity in the fungal isolates under Pb stress

Fig. 4: MDA activity in the fungal isolates under Pb stress

Fig 5

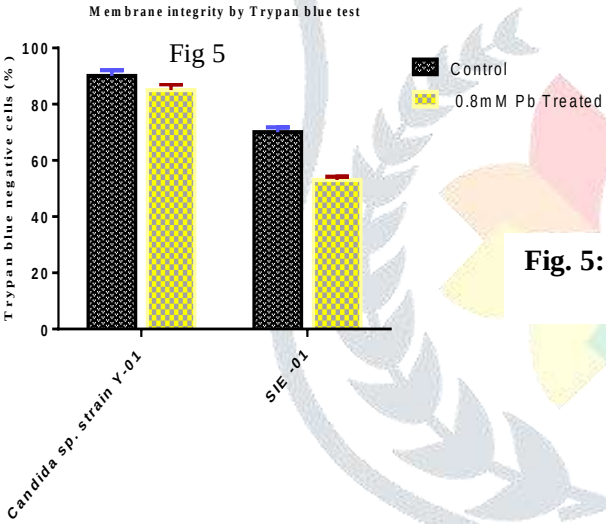

Fig. 5: Membrane integrity analysis in fungal isolates under Pb stress.

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