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Biochemical, Enzymatic and Energy Alterations Observed in *Saccharomyces cerevisiae* Subjected to Oxidative Stress

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Abstract: The objective of this work is to characterize the adaptive response of yeast (*Saccharomyces cerevisiae*), in protein, carbohydrate, enzymatic (CAT) and respiratory against cadmium (Cd) and zinc (Zn) in combined treatments in the presence of calcium and oxidative stress that results. The concentrations of cadmium and/or zinc in the presence or absence of calcium were used (0.1 mM Cd^{2+}/0.1 mM Ca^{2+}, 0.1 mM Cd^{2+}/0.1 mM Zn^{2+}, 0.1 mM Ca^{2+}/0.1 mM Zn^{2+} and 0.1 mM Cd^{2+}/0.1 mM Ca^{2+} / 0.1 mM Zn^{2+}). The obtained results showed that the determination of enzyme activity (catalase) is strongly stimulated particularly treatments combined (mixture of two metals), however, reduced the rate of total protein was observed with combination therapy, it is near 22% for treatments Calcium / Zinc and Cadmium / Calcium / Zinc. Meanwhile, the rate of carbohydrate has been a sharp decrease in cells treated with Cd^{2+}/Ca^{2+}/ Zn^{2+}. It is about (83%), it is (34%) for the treatment Cd^{2+} / Ca^{2+} while it decreases near (48%) for the treatment Cd^{2+}/Zn^{2+}. For treated Ca^{2+} / Zn^{2+} and Cd^{2+}/Ca^{2+}/Zn^{2+} inhibition of respiratory activity is observed with an oxygen uptake of about (42%). In conclusion, cadmium exerts oxidative stress led to many alterations of biochemical parameters (enzymatic and respiratory).

Key words: *Saccharomyces cerevisiae* · Oxidative stress · Detoxification · Cadmium · Zinc · Calcium

INTRODUCTION

Xenobiotics found in the environment are causing harmful effects in living organisms. However, we are still far from knowing all the mechanisms of action of pollutants and responses put in place in organisms to survive in a polluted environment. In this work, we used the yeast *Saccharomyces cerevisiae* as a model alternative to highlight responses to stress induced by mixtures of cadmium and zinc; in fact, this yeast is often used as an indicator of pollution [1, 2].

Studies show that metals are capable of inducing various pathologies. Some are purely toxic to living things (cadmium), others are essential to the organization and maintenance of biological functions, generating still toxic effects after a certain threshold concentration (zinc).

Cadmium belongs to the second category (toxic) elements for high toxicity to the biological community key known to date, hence the need for detoxification or excretion of the organism [3, 4]. Many toxic symptoms are caused by the excess of Cd (growth retardation, inhibition of photosynthesis, induction and inhibition of enzymes, altered stomatal action and influx of cations and generation of free radicals [5]. Cadmium can inhibit the growth of fungi, Saccharomyces cerevisiae [6] and alter calcium homeostasis, which is a universal and versatile intracellular messenger [7]. Cadmium poisoning may increase serum calcium via the G protein coupled factors bonding metals [8]. Cadmium can induce the appearance of reactive species of oxygen (ROS) by indirect mechanisms. It is also described as an inhibitor of DNA repair [9]. The metal may have significant toxicity by interference with essential metals such as iron, copper or zinc. The disruption caused by cadmium lead to several adverse consequences for the cell.

However, zinc (Zn) is defined as a metal that occurs physiologically in major metabolic pathways, either as cofactor or as a constituent of the structure of enzymes such as alkaline phosphatase, glutamate dehydrogenase or superoxide dismutase (anti -oxidizing). It acts as a

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structural component of many transcription factors, which explains its pivotal role in controlling gene expression [10]. The number of zinc enzymes is impressive. However, it can inhibit enzymes such as protein tyrosine phosphatase and caspase-3 [11]. Zinc is also involved in growth and cell proliferation [12, 5], in the immune response, in reproduction [13, 14], in hormone metabolism, or in protection against free radicals [11]. The cellular zinc homeostasis is a balance between systems allowing its import into the cell system output to external storage systems and exchange including metallothioneins (MTs) STD control homeostasis of zinc and copper in storing or exchanging these micronutrients with other proteins [15].

The general or localized excess of zinc is responsible for its toxic effect. Zinc may thus move from a role in antioxidant prooxidant role: in a free state, the latter would be responsible for the indirect formation of free radicals. In other cell types, it induces the formation of H_2O_2. Zinc excess also appears to inhibit the glutathione reductase/peroxidase and major enzymes responsible for antioxidant defense [16, 17, 18]. Excess Zinc is also accompanied by a decrease of GSH and oxidative stress involved in neurodegenerative diseases [19, 20]. It can also cause decreased immune function and respiratory depression [21].

In this work, we are interested in the behavior of yeast (Saccharomyces cerevisiae), vis-à-vis a type of metal pollution. This study concerns aspects protein, carbohydrate and respiratory enzyme. A final aspect of this work concerns the influence of second xenobiotic zinc applied in combined treatment with cadmium in the presence of calcium on the behavior of Saccharomyces cerevisiae.

MATERIAL AND METHODS

Cultivation of Yeast: The biological material used is fungus unicellular eukaryotes: the yeast *Saccharomyces cerevisiae*. This strain was isolated from the culture medium YPDA (10 g/L glucose, 10 g/L yeast extract, 10 g/L Bactopeptone and 20 g/L agar).

Treatment of Yeast: The chemicals used are two heavy metals: cadmium chloride ($CdCl_2$) and zinc sulfate ($ZnSO_4$), plus calcium chloride ($CaCl_2$).

We tested several combinations of these compounds to assess the impact, the combined concentrations are: 0.1 mM Cd^{2+} / 0.1 mM Ca^{2+}, 0.1 mM Cd^{2+} / 0.1 mM Zn^{2+}, 0.1 mM Ca^{2+} / 0.1 mM Zn^{2+} and 0.1 mM Cd^{2+} / 0.1 mM Ca^{2+} / 0.1 mM Zn^{2+}.

Determination of Biomarkers

Determination of Total Protein: The method used for determination of total proteins in yeast is the method of Bradford (1976) using BAS (Bovine Albumin Serum) as standard. It is a colorimetric method based on the absorption of the dye Coomassie Blue G250, in solid, the basic groups and/or aromatic proteins followed by spectrophotometric measurement at 595 nm.

Determination of Total Carbohydrate: The determination of carbohydrates for the different treatments and for control cells is achieved by experimental protocol.

Measurement of Catalase Activity (CAT): The method used for determination of catalase activity (CAT) in yeast is the method of Regoli and Principato (1995). The catalase activity (CAT) is measured using a spectrophotometer (Jenway 6105) at a wavelength ë=240 nm by the change in optical density OD following the dismutation of hydrogen peroxide (H_2O_2) by reacting in 100mM phosphate buffer (pH7.5) for 1 minute presence of 20µl of supernatant of the suspension of yeast and an incubation temperature of 25 °C.

Measurement of Respiratory Activity: The respiratory activity of yeast is measured by the method of Djebar and Djebar, 2000. This method relies on the use of an oxygen electrode, called Clark electrode.

Statistical Analysis: The statistical analysis was performed by Student t test used to compare between two samples (control and treated) at a significance level above 95% ($p < 0.05$) and by calculating correlation coefficient Pearson r. This test is performed using the analysis software statistical processing of data: Minitab version 16.1.0.

RESULTS

Mean Levels of Total Protein: A slight increase of total protein (about 10%) was noted for cells treated with 0.1 mM Cd^{2+} in the presence of 0.1 mM Ca^{2+}. However, a reduction in the rate of total protein is observed in yeast subjected to other treatments combined, it is nearly 22% for treatments Ca^{2+} / Zn^{2+} and Cd^{2+} / Ca^{2+} / Zn^{2+}. The statistical study highlights differences very highly significant ($p < 0.001$) between the rate of total protein of control cells and treated with Cd^{2+} / Ca^{2+} / Zn^{2+}, Cd^{2+} / Ca^{2+} and Cd^{2+} / Zn^{2+}.

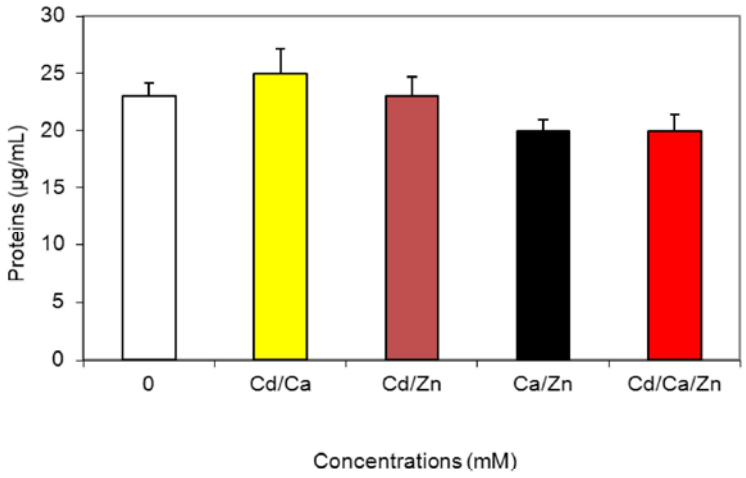

Fig. 1: Effects of combined treatment (Cd^{2+} / Ca^{2+} / Zn^{2+}) on changes in mean levels of total protein in *Saccharomyces cerevisiae*

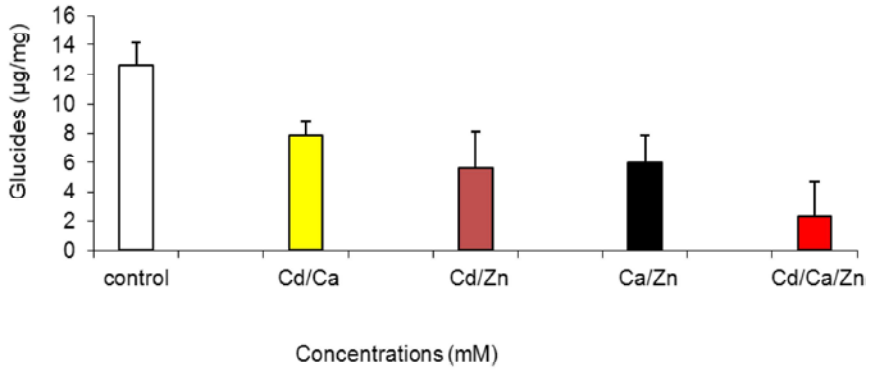

Fig. 2: Effects of cadmium on changes in mean levels of total carbohydrates in *Saccharomyces cerevisiae*

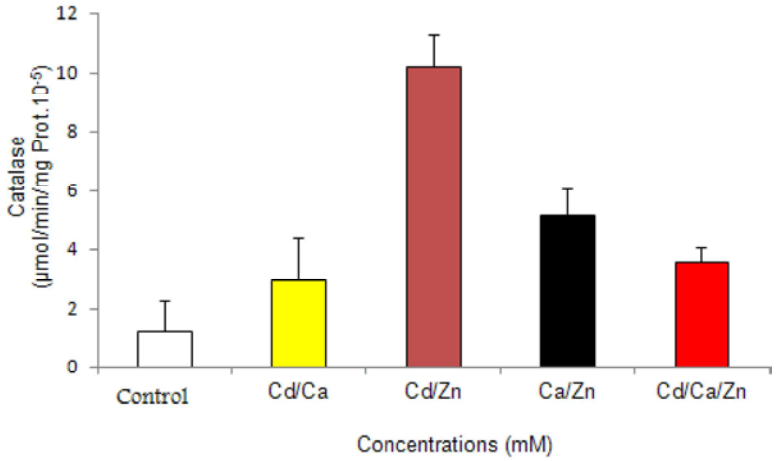

Fig. 3: Changes in catalase enzyme activity based on combined treatments in *Saccharomyces cerevisiae*

Average Levels of Total Carbohydrates: Regarding the rate of carbohydrate, the latter decreases with different concentrations of cadmium, it increases from 8.5 mg/mg to 1.85 mg/mg. The rate of total carbohydrate recorded a decrease (approximately 85%) in cells treated with especially high concentrations of Cadmium (5 mM and 10 mM) and this compared with controls, it is of the order of 12.65 mg/mg. The statistical study shows that

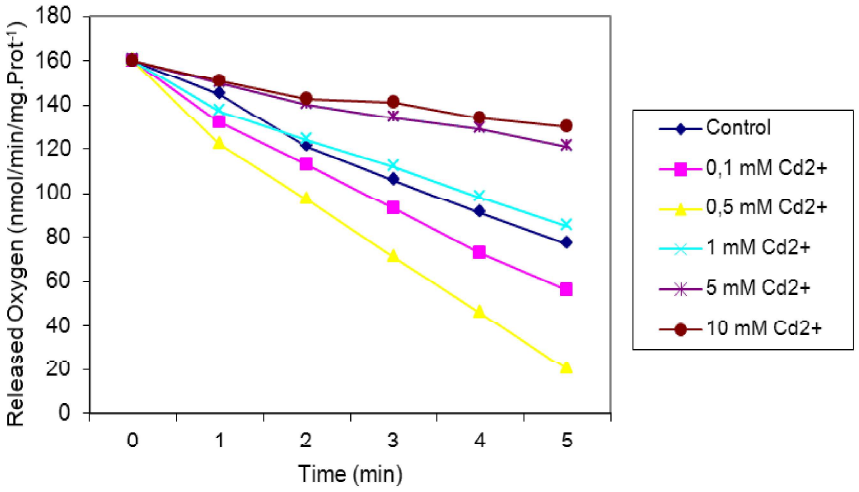

Fig. 4: Changes in respiratory activity of Saccharomyces cerevisiae as a function of different concentrations of cadmium

differences between treated and control yeasts were not significant ($p > 0.05$) and the correlation observed between the rate of total carbohydrate and the different treatments of cadmium remains high ($r = 0.962$; $p < 0.05$).

The Enzymatic Activity of Catalase: The measurements of catalase enzyme activity showed a slight increase in the Yeast treated with Cd^{2+} / Ca^{2+}, Cd^{2+} / Ca^{2+} / Zn^{2+} and Cd^{2+} treatment / Ca^{2+} and Ca^{2+} / Zn^{2+}, it is about (10%). Meanwhile, there has been a sharp increase in catalase activity in the treatment Cadmium / Zinc (80%), it is 1.23 µmol. Statistical analysis shows that differences recorded between control and treatment were significant ($p < 0.05$).

Respiratory Activity: There is evidence that yeast treated with Cd^{2+} / Ca^{2+} and Cd^{2+} / Zn^{2+} have a respiratory activity with a high oxygen consumption higher (56%) compared with control cells. For cells subjected to treatment Ca^{2+} / Zn^{2+} and Cd^{2+} / Ca^{2+} / Zn^{2+} inhibition of respiratory activity is observed with an oxygen uptake of about (42%). The statistical study shows that differences between yeast and witnesses are treated very highly significant ($p < 0.001$).

DISCUSSION

All results obtained in this work shows that combined treatments are causing stress, confirmed by the values of changes biomarkers measured in *Saccharomyces cerevisiae*. Indeed, we find that the presence of calcium in the treatment of yeast by Cadmium causes a slight increase in the rate of survivors, alongside the presence of zinc has resulted in a plateau close to that obtained with control cells. These observations confirm those reported by [22] where it is established that zinc prevents the inhibitory action of cadmium observed in macrophyte. Zinc is an antagonist of cadmium while a micro-essential element in nutrition [23]. Another mechanism by which cadmium interferes with calcium homeostasis is linked to its ability to modulate the extracellular calcium receptor [24]. Thus, Cd may profoundly affect cell functions comprising the system of detection of extracellular calcium.

The determination of total proteins n cells subjected to treatment with cadmium showed that *Saccharomyces cerevisiae* is very sensitive to cadmium. The presence of calcium causes a slight increase and it is the same for zinc. Indeed, some researchers show that cadmium and copper caused a fall in average total protein, in parallel, shows that the behavior of microorganisms treated by zinc is bound concentrations used. The physiological concentrations are essential for living organism cons by high concentrations of zinc tend rather to interfere with cations and thus greatly modify enzymatic activities sometimes indispensable to the functioning of certain organelles. The general or localized excess of zinc is responsible for its toxic effect. Zn can thus move from a role in antioxidant peroxiding role, in a free state, the latter would be responsible for the indirect formation of free radicals. The sequestration of Zn excess by mitochondria would lead to its dysfunction characterized by prolonged release of ROS [25]. In other cell types, Zn has been identified as inducing the formation of H_2O_2 [26]. The excess Zn also appears to inhibit glutathione reductase

and peroxidase enzymes responsible for major and antioxidant defenses [17, 18]. This inhibition occurs mainly through interaction of Zn with thiol groups located in/near the active sites. These observations are supported by other data that suggest that exposure of liver cells to 30 µM of Zn would lead to an inhibition of enzymes glutathione (GSH). This inhibition results in the decrease of GSH, increased GSSG and an imbalance in favor of oxidative free radicals [27, 28]. The excess of Zn is accompanied by a decrease of GSH and oxidative stress involved in neurodegenerative diseases [19, 20]. [29] show that Zn may induce neuronal death via production of ROS. These authors have observed an increase in lipid peroxidation after incubating the cells with concentrations of Zn = 40 µM.

Regarding changes in average rates in carbohydrates, they experience a strong disturbance and these both in the presence of cadmium in the presence of Calcium and Zinc. This result is in perfect agreement with those reported by [30] when in fact the treatment with cadmium grain pea causes dysfunction of the mobilization of carbohydrate reserves. The mechanism involved in this case remains unsolved.

Among the antioxidant defenses of the cell, we note that the catalase activity information on the degree of cellular alteration, our results show that the catalase activity measured in the presence of cadmium is stimulated so important. Contrarily, there is a slight increase in the presence of Calcium and Zinc. The combined treatments particularly the association Cd/Zn show cons by a strong stimulation. This result is very important in that it is actually reported that the presence of zinc causes a stimulation of antioxidant enzyme activities particularly in the presence of Cadmium [22]. Other recent scientific contribution to our results [31]. It is well accepted that to avoid damage induced no intracellular cadmium, cells respond to heavy metal contamination by the induction of transcription of genes that encode proteins of "defense" or "repair". These proteins can sequester metals to neutralize their toxic effects, fight against the formation of reactive species of oxygen and repair (if they are not inhibited by cadmium) damage at the DNA level or renature or degrade misfolded proteins. Contamination by cadmium may result in the induction of several genes in response to stress such as genes coding for metallothioneins (MTs) [32], heat shock proteins (HSPs, Heat Shock Proteins) [33], genes involved in oxidative stress response or the synthesis of glutathione [34]. In general, the protein thiol group plays a key role in cellular defense against cadmium toxicity. This is particularly true of proteins with MTs 1/3 amino acids are cysteine. They can set their cadmium thiol groups and cause a decrease in the concentration of Cd can interact with other cellular targets. The generation of denatured or abnormal proteins by cadmium in reaching their thiol group or by substituting zinc these proteins is considered to signal induction of HSPs [35]. Because metallothioneins, reduced glutathione (GSH) can bind cadmium and prevent adverse interaction with target cells [36]. Moreover, the redox cycle of glutathione (GSH), whch involves glutathione peroxidase and glutathione reductase plays an important role in the detoxification of ROS generated by cadmium. Indeed, an increased synthesis of GSH protects the cell toxicity of cadmium (Chin and Templeton, 1993). In general, the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase remained basal [37]. However, an increase of enzyme activity is observed at a cadmium contamination of cultured cells or animals [38]. Disturbances recorded at the level of respiratory metabolism in general show that high concentrations of cadmium inhibit the respiration of Saccharomyces cerevisiae cells while low levels tend to produce mild stimulation. The measurement of cellular respiration (by measuring the oxygen dissolved in the middle) is an indicator of dysfunction of mitochondrial respiratory system (energy) of *Saccharomyces cerevisiae,* in fact, it is a sensitive target specific but different pollutants. The cyanide ions inhibit cellular respiration, while 2,4-dinitrophenol stimulates. The mercury and copper ions inhibit cellular respiration after having driven for several minutes. Saccharomyces cerevisiae is very sensitive to the presence of cadmium ions. A minute and a half of yeast contact/Mercury sufficient to inhibit cellular respiration [1]. This could be explained by the fact that low concentrations of cadmium are rapidly vacuolated and/or so diluted in the living organism (*Saccharomyces cerevisiae*), which significantly reduces their toxic effects. In this activity may be added stimulation of antioxidant enzymes involved in detoxification such as catalase, which results in this case by stimulation of the respiratory activity of yeasts. Alongside the high concentrations of cadmium may be accompanied by a change due to the immediate environment of yeast (Saccharomyces cerevisiae) which induces a massive entry of ions (probably K^+). This influx of K^+ and Cd^{2+} destroying the electrochemical potential ($\Delta\mu H^+$) and membrane ($\Delta\Psi$) initially in equilibrium with the external environment of yeast, which results in an inhibition of respiratory activity of cells of *Saccharomyces cerevisiae*.

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