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Effect of zinc on growth, metabolism and activity of antioxidant enzymes in the yeast *Saccharomyces cerevisiae*

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ABSTRACT

This workaims the study ofeffect of zinc on a microorganism *Saccharomyces cerevisiae*.The main results show that the presence of zinc significantly affect the growth of yeast. On the metabolic level we note a significant increase in protein and a decrease in carbohydrates.Regarding the biomarkers, we have identified a significant decrease in glutathione (GSH) levels and the activation of the detoxification enzyme which results in a significant increase of glutathione –S- transferase (GST) and Catalase activity.

Key words: *Saccharomyces cerevisiae,* Zinc, biomarkers, GSH, GST, Catalase.

Introduction

Living organisms are continuously exposed to exogenous and endogenous toxic chemical species, which may cause adverse and sometimes lethal effects. The ability of living organisms to survive the risk posed by such compounds represents a fundamental biological adaptation for survival. Different strategies have been adopted by cells to counter the effect of toxic compounds and their metabolites. Defense mechanisms are usually general, rather than specific for a given chemical or organism. Among the defense mechanisms, such as sequestration and binding, catalytic biotransformation was evolved as a crucial mode of protection against toxic chemical species (Sheehan, D., 2001).

Cells possess a broad ensemble of enzymes capable of transforming a wide range of different chemical structures and functionalities. The enzymatic detoxification of xenobiotics has been classified into three distinct phases, which act in a tightly integrated manner. Phases I and II enzymes catalyze the conversion of a lipophilic, non-polar xenobiotic into a more water-soluble and therefore less toxic metabolite, which can then be eliminated more easily from the cell. Phase I of detoxification is mainly the result of action by the cytochrome P450 system and mixed function oxidases (Ishikawa, T., 1997). These proteins are responsible for a wide range of reactions, of which oxidations appear to be the most important (Guengerich, F.P., 2001). Phase II enzymes catalyze the conjugation of activated xenobiotics to an endogenous water-soluble substrate, such as reduced glutathione (GSH) or uridinediphosphate (UDP)-glucuronic acid. Quantitatively, conjugation to GSH, which is catalyzed by glutathione S-transferases (GSTs), is the major phase II reaction in many species and also generally like other eukaryotes and Gram-negative aerobic yeast Saccharomyces cerevisiae has a non-enzymatic antioxidant, glutathione (γ-L-glutamyl-L-cystinylglycine), which can a from participating in the antioxidant defense as a source of reducing equivalents, and secondly to serve as a cofactor for certain enzymes involved in protection against oxidative stress (Ernst, R., 2005).

Zinc is one of the micronutriments essential for normal growth and development of plants, as it is known to be required in several metabolic processes (Prasad, M.N.V., 1999). However, the presence of zinc at higher concentration retarded growth and development of plants,by interfering with certain important metabolic processes (Prasad, M.N.V., 1999).

The objective of this work is to study the effects of oxidative stress induced by heavy metal (Zinc) on a micro-organism bio accumulator of metals, the yeast *Saccharomyces cerevisiae.*

Materials and Methods

Biological Material:

The biological material used is a microorganism unicellular eukaryote yeast *Saccharomyces cerevisiae*. The yeast *Saccharomyces cerevisiae* is grown in the culture medium glucose yeast extract (20g glucose, 5g yeast extract and 1000 ml of distilled water, pH 5.6) (Larpent, J.P., 1997).

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Chemical material:

The chemical material used is a chemical heavy metal: Zinc sulfate (ZnSO4) "Panreac, 99%." Processing mode: different dilutions ($ZnSO_4$) is the following concentrations: 0.05mm, 5 mM, 10 mM are prepared from a stock solution of 10 mM, the different treatments of zinc are performed in culture media from a culture of 24H (Guelfi, A., 2003; Tatina, T., 2007).

Measured parameters:

Kinetics of growth:

The **Kinetics of** growth of yeast is done by measuring the optical density (OD)at wave length λ= 620nm (Pol, D., 1994), extraction of metabolites was performed according to the method of Shibko *et al*., (1966) with which the proteins were quantified by the method of Bradford (1976), the determination of carbohydrates is performed according to the method of Duchateau & Florkin (1959), glutathione is estimated by a method Weckberker and Cory (1988), glutathione-S-transferase (GST) was measured at 340 nm using the method of Habig *et al*. (1974), The catalase activity (CAT) is followed directly at 240 nm according to the method of Regoli and Principato (1995).

Statistical analysis:

The results are shown as mean ± standard error, the results are compared by nonparametric Kruskal-Wallis by the software MINITAB Version 14.0, the significance level chosen is $p < 0.05$ (Dagnelie, P., 1999).

Results:

Effect of zinc on the growth of Saccharomyces cerevisiae:

Figure (01) shows the evolution of yeast growth with times, we note that in the treated concentration (0.05mM) Zinc, growth tends to increase with time and reaches its maximum to 90mn. In contrast, in the treated concentrations (5 mM and 10 mM), we observe a decrease in the growth of a dose-dependent manner after 90 mn.

Statistical analysis shows that there is no significant difference between control cells and treated with the concentration (0.05mM) and a significant difference between control cells and treated with the concentration (5 mM). Noted also a difference very highly significant between control cells and those treated with the concentration (10 mM).

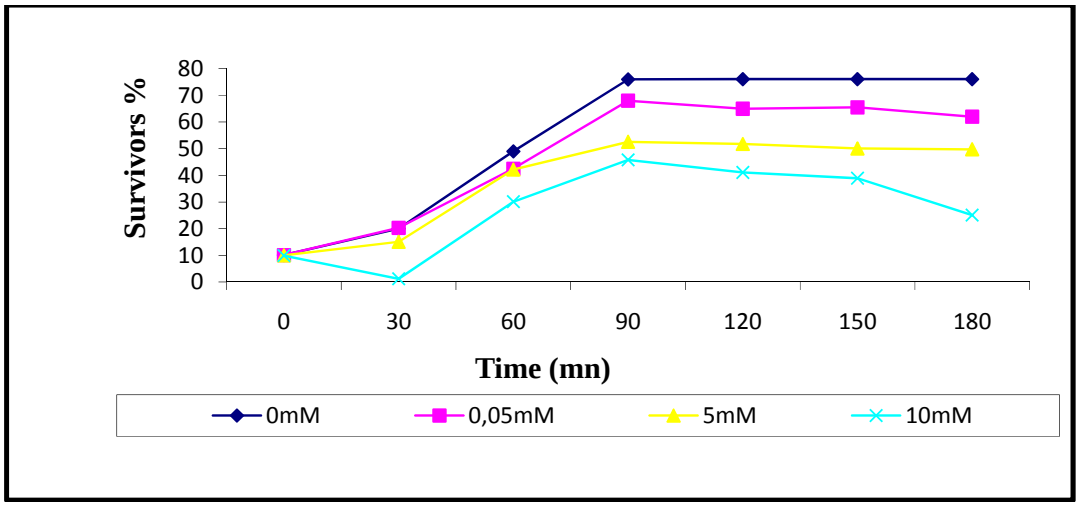

Fig. 1: Effect ofzinconthe growth kinetics of*Saccharomycescerevisiae.*

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Effect of Zincon themetabolite levels(total proteins,totalcarbohydrates):

Table (01) shows variations intotal proteinand carbohydratesin yeastin the presence of Zinc, we find that in the Treaties, the rate of total proteintends to increasein a dose-dependent and highly significant compared to witnesses for the concentration (5 mM) and isvery highly significant for the concentration (10 mM). However, the rate of carbohydratetends to decreasein a dose-dependent and significant for the treated concentration (0.05m Mhighly significant for the treated concentration (5 mM) and very highly significant for the treated concentration (10 mM) compared to controls.

Table 1: Effect of Zincon changes inmetabolites (total protein and total carbohydrate) in *Saccharomycescerevisiae*.

Metabolites (µg/ml of culture)	Concentrations of Xenobiotic (Zinc)			
	0mM	0.05mM	5mM	10mM
Proteins	5.1±05	8.2±0.20	11.50±0.34**	16.01±0.26***
Carbohydrates	32.2±0.4	25.01±0.3*	17.8±0.2**	12.35±0.15***

Effect of Zinc on glutathione (GSH):

Figure (02) shows the changes of GSH in the presence of zinc in the yeast *Saccharomyces cerevisiae.* And in the presence of xenobiotic rate GSH slightly decreased in cells treated with the concentration (0.05mm) compared to controls, but the rate of GSH decreases in a manner highly significant ties among treated concentrations (5 mM) and (10 mM) by providing witnesses.

Effect of Zinc on glutathione S-transferase (GST):

Figure (03) shows the changes in GST activity in the presence of zinc in the yeast *Saccharomyces cerevisiae.* And in the presence of xenobiotic GST rate tends to increase in a dose-dépendante. Analysis revealed a statistical difference between the control and treated is significant for the concentration (0.05mm), a highly significant difference in the concentration (5 mM) and a highly significant difference for those highly processed by the concentration (10 mM).

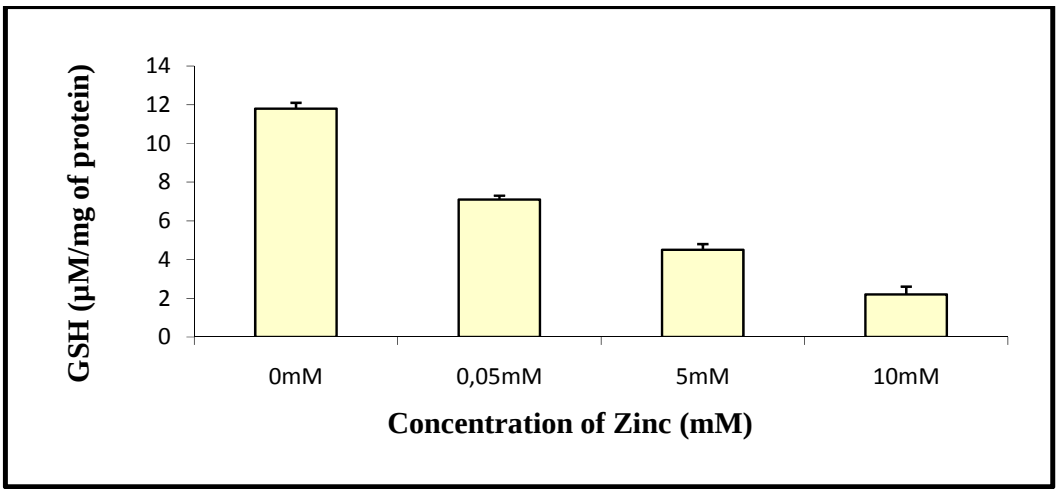

Fig. 2: Effect of Zinc on GSH levels in *Saccharomyces cerevisiae.*

Effect of Zinc on Catalase activity:

Figure (04) shows the variations in catalase activity under the effect of zinc in the yeast *Saccharomyces cerevisiae.* Our results show that the presence of xenobiotic Catalase activity increased dose-dependent manner for cells treated with concentrations (0.05mM and 5 mM), then she slightly lower in the treated concentration (10 mM) of zinc.Statistical analysis revealed a significant difference between the control and the treated concentration (0.05mM) and very highly significant difference for those treated with concentrations (5 mM and 10 mM).

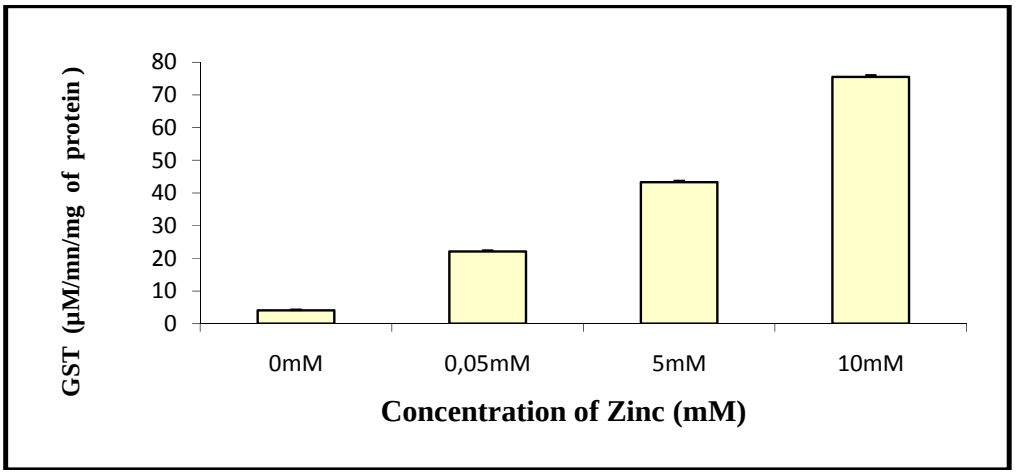

Fig. 3: Effect of Zinc on the GST activity in *Saccharomyces cerevisiae*.

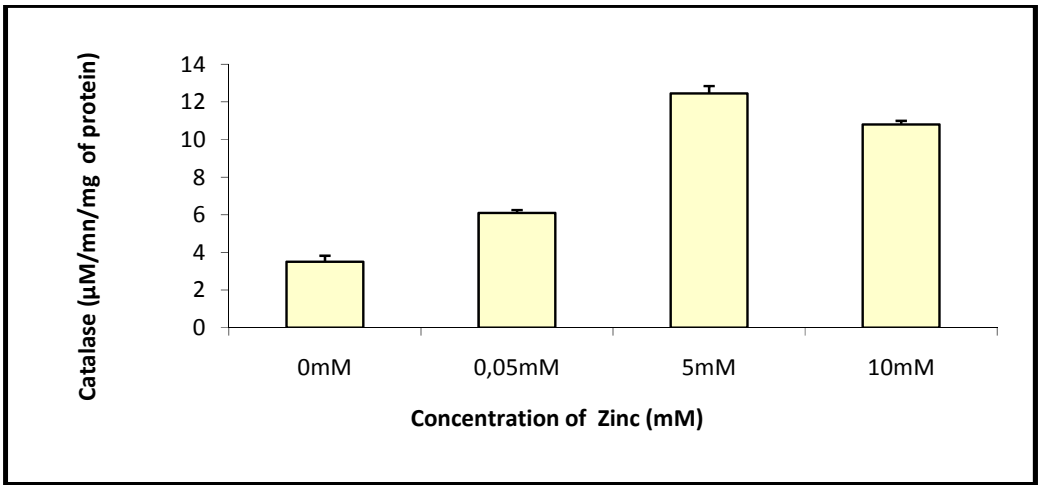

Fig. 4: Effect of Zinc on Catalase activity *Saccharomyces cerevisiae*.

Discussion:

Numerous studies have shown that most heavy metals are considered true toxic agents, disrupting certain enzyme systems and also the metabolic and physiological activities in humans and animals (Iscan, M., 1994). Metals generate oxygen radicals such as the hydroxyl radical OH potent toxic at the cellular level that are at the origin of the phenomenon known as the more general term "oxidative stress", heavy metals can induce a state of general stress, resulting in reduction their ability to adapt to hypoxia (Le Bras, G.J., 2007). The Xenobiotic tested is toxic to the yeast Saccharomyces cerevisiae, this toxicity is manifested primarily by an inhibition of cell growth, these results are consistent with the work of Einicker Lamas *et al*. (2002) who studied toxicity of zinc and copper on chlorophyll algae Euglena gracilis.Il is weak as well as the work of Fukusshina andal. (1979) who studied the toxic effects of heavy metals on paramecium, they put evidence that its growth was inhibited in the presence of certain metals (Fukusshina, S., 1979).

In our work, we demonstrated that the protein levels in a dose-dependent manner in the presence of zinc, these results point in the same direction as those of Peccini andal. (1994) who showed a significant increase in total protein under the effect of a chemical stress among ciliates. On the other hand (Peccini, E., 1994), concerning the evolution of carbs, we noted that this rate decreases by a dose-dependent manner in the presence of Zinc, this decrease was due to carbohydrate oxidation in the presence of metal ions leading the release of aldehydes and hydrogen peroxide (Nzengue, Y., 2008). Indeed, carbohydrates are the primary sources and immediate energy in the stress condition, the reserves of carbohydrates are exhausted to satisfy the energy demands (Leheninger, A.L., 1978). In the stress condition, metabolism may represent a potential target for the toxicity of heavy metals (Canesi, L., 1998), declining energy reserves due to a high metabolism or structural

changes and permeability of cell membranes may explain this effect (Møller, V., 1994). In our work, we demonstrated a dose-dependent decrease of GSH in the presence of zinc in the yeast *Saccharomyces cerevisiae*, this depletion is due to detoxification mechanism activated in cells via conjugation reactions with glutathione (Nzengue, Y., 2008). On the other hand, according to the study by Christie and Costa (1984) metals that cause the oxidation of GSH are (Cu, Co, Mn, Fe and Cr) (Christie, N.T., M. Costa, 1984), while stable complexes with GSH are formed by the (Zn, Cd, Hg, Pb and Ni) the latter reaction may explain the decrease of glutathione (Halliwell, B., J.M.C. Gutteridge, 1985; Stohs, S.J., D. Bagchi, 1995). According Canesi Viarengo and (1997), reduced glutathione content is mainly correlated with a decrease in the activity of the glutamyl cysteine synthetase, the latter involved in the biosynthesis of GSH. These results are in the same direction as those of (Grant *et al*., 1998).

Our results show a significant increase in the rate of GST, this increase is a response to oxidative stress caused by the presence of a heavy metal in yeast (Farombi, E.O., 2007). Biotrans formation enzymes are among the first to respond to the presence of a xenobiotic in a living organism. This increase indicates a high rate of xenobiotic conjugation with glutathione (Peršić, A., 2004). This is in agreement with the work of (Lea *et al*., 2002) showed an induction of GST in *Sacchaumofficinarum* after treatment with cadmium (Lea, P.J., 2002).

On the other hand our results showed an increase in catalase activity in the yeast *Saccharomyces cerevisiae* treated with different concentrations of zinc, due to the increased antioxidant activity in cells. We found in our study that the significant effects of catalase activity were observed at lower concentrations the dice and this may be due to the sensitivity of this biomarker, indeed catalase is considered one of the most biomarkers sensitive oxidative stress (Livingstone, D.R., 2001), especially with respect to chemical pollutants in the aquatic environment (Regoli, F., 2003). Our results are in agreement with the work of Guelfi *et al*. (2003), which have demonstrated increased activity in the fungus *Aspergillusnidulans* after treatment with cadmium. These results point in the same direction as those of (Lu *et al*., 2009) showed a significant increase in catalase activity and *Stenotrophomonasmaltophilia* WZ2 *Escherichia coli K12* (Lu, Z., 2009).

Conclusion:

It is clear that the species *Saccharomyces cerevisiae* is sensitive to the presence of Zinc, this sensitivity is manifested by a disturbance of cell growth, accompanied by increased production of free radicals, which resulted in a significant increase in protein, reduced carbohydrate and a significant decrease of glutathione, and the initiation of a battery of enzymatic processes such as GST and catalase activity known for their role in detoxification.

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