HEAT-SHOCK AND TITANIUM DIOXIDE NANOPARTICLES DECREASE SOD AND GLUTATHIONE ENZYMES ACTIVITIES IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction – It is well-known that the majority of living organisms depend on oxygen for survival. However, organisms also had to evolve a multitude of enzyme antioxidant defences as superoxide dismutase (SOD1, SOD2), glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR), glutathione peroxidase (GPx), and catalases (CTT1, CTA1) as well as non-enzyme defences as glutathione, to protect their cells from toxicity of reactive oxygen species (ROS). Exposure of living organisms to xenobiotic can also induce significant generation of ROS. Failure of cell antioxidant defences to prevent ROS accumulation inevitably results in oxidative stress. This potentially causes severe oxidative damages in vital biomolecules, thus compromising cell viability. Yeasts can provide a significant contribution to our understanding of oxidative stress, and its consequences on cell death, because its cellular structure and functional organization share many similarities with plant and animal cells. Although ROS accumulation in yeast generally results from cell respiration, environmental stress stimuli can be also another important source. Despite the intensive use of engineered nanoparticles (NPs) in various consumer and industrial products, data on their potential hazards are still rare and mechanisms of action only partially understood. In addition, NPs as titanium dioxide nanoparticles (TiO_2-NP) possessing unique physicochemical characteristics such as high specific surface area, high reactivity, and rapid diffusion, which differ from bulk materials of the same composition (TiO_2). On the other hand, yeast response to ROS (H_2O_2) or the toxicity of NPs depends on environmental conditions as temperature. Consequently, the aim of this work was to evaluate the antioxidant response of *Saccharomyces cerevisiae,* grown in presence of glycerol or glycerol and glucose, to 5 µg/mL TiO_2-NP in heat-shock conditions.

2. Experimental – TiO_2-NP (size <100 nm, Sigma) stock suspensions were prepared by sonication (130-Watt) for 30 min. Bioassays were performed in 250-mL Erlenmeyer flasks containing 100 mL of YEPG basal medium (1 % yeast extract, 2 % peptone, 3 % glycerol). Culture flasks were inoculated using fresh culture of wild-type *Saccharomyces cerevisiae* UE-ME_3 and shaken 150 rpm, at 28 ºC. At exponential growth phase (OD=0.8) was added 2% glucose (YEPGD medium). After 100 min was added TiO_2-NP stock solution to obtain a final concentration of 5 µg/mL. Yeasts were allowed to grow 100 min at 28 ºC or 40 ºC (heat-shock, ST). Flasks lacking glucose or NPs served as controls. Biomass was quantified by dry weight. Post-12000 *g* supernatants were used for determination of contents in GSH, GSSG and ROS by fluorescence as well as enzyme activities GR, GPX, G6PD, CTT1 and SOD1by spectrophotometry. Post-12000 *g* pellets were used for determination of enzyme activities CTA1 and SOD2. All values were presented as mean of five independent experiments ± SEM. The statistical analysis of results were performed by ANOVA I and Duncan test to determine significant differences ($p < 0.05$) between treatments, using SPSS for Windows, version 22, licensed to University of Évora.

3. Results and Discussion - The results showed that biomass and levels of ROS and GR activity in the cells grown in YEPGD medium were higher than those detected in cells grown in YEPG basal medium. Furthermore, cells grown in medium YEPGD exhibited lower levels of glutathione, GSH and MDA contents, CTT1 activity than those detected in yeasts grown in basal medium YEPG. *S. cerevisiae* grown in YEPGD and 5µg/mL TiO_2-NP in heat-shock conditions showed a growth inhibition to the levels near of cells which used only glycerol as carbon source. Additionally, it was also determined a decrease in the GSH contents, GSH/GSSG ratio, G6PD, GR, GPx, SOD1 and SOD2 activities as well as an increase in ROS content and CTA1 activity, relatively to the controls.

4. Conclusions - TiO_2-NP in heat-shock conditions cause oxidative stress in *S. cerevisiae* grown in presence of glycerol or glycerol and glucose, decreasing antioxidant defences as SOD and glutathione enzymes.