Abstracts

9th Iberian and 6th Iberoamerican Congress on Environmental Contamination and Toxicology

The environmental research: essential for sustainability

CICTA 2013

Valencia-Spain | 1 to 4 | July | 2013

Nanoparticles of titanium dioxide modulate the response to temperature by key enzymes involved in pyruvate availability in cytosol and mitochondria of *Saccharomyces cerevisiae* BY4741

J. Capela-Pires*, R. Ferreira, I. Alves-Pereira*

ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Dep. Química, ECT, Universidade de Évora, Ap.94, 7002-554 Évora, Portugal

*Presenting author contact: **iap@uevora.pt**

Nanotechnology can be used to obtain materials at nanoscale (<100 nm) with new physicochemical and structural properties which depend on particle size and, probably, may trigger new biological effects. As *Saccharomyces cerevisiae* is an excellent model for study molecular and cell biology responses is growingly used in the toxicological evaluation of chemicals, such as heavy metals or nanoparticles of metal oxide due increasing use of these materials in consumables as cosmetics and textiles. The malate dehydrogenase (EC 1.1.1.37, MDH2) and malic enzyme (EC 1.1.1.38/39, ME1) of *S. cerevisiae* catalyze the oxidative decarboxylation of L-malate to pyruvate and CO2 coupled to reduction of NAD(P)+ in NAD(P)H of cytoplasm and mitochondria. These enzymes are part of metabolic crossroads that are implicated in regeneration of pyruvate, thereby contributing to the functionality of the citric acid cycle and generation of reducing equivalents as NADPH or NADH, required for *de novo* fatty acid biosynthesis and antioxidant response or respiratory chain. Hence, the main purpose of this work was to evaluate how nanoparticles of titanium dioxide modulate the effects of temperature on pyruvate availability in *S. cerevisiae* BY4741, a EUROCAST strain. Yeast (106 cells mL-1) at mid-exponential phase were inoculated in YEPD medium with 2% (w/v) glucose and allowed grown in a water bath, with orbital stirring at 25, 28, 30 or 40ºC, during 200 min in absence or presence of 0.1 or 1.0 µg/mL TiO2-NP. Samples from each treatment, suspended in 10 mM phosphate buffer, pH 7.0 were lysed by sonication and centrifuged at 12,000 *g* during 20 min, at 4ºC. Aliquots of supernatant and pellet were stored at -20ºC for later use. Protein contents in the cell fractions as well as enzyme activities MDH2, G6PD and ME1 were determined in the post-12,000 *g* supernatant or pellet by spectrophotometry. ROS and MDA contents were estimated in the post-12,000 *g* supernatant by fluorimetry. Nanoparticles of titanium dioxide (<100 nm) were purchased from Sigma-Aldrich. Statistical analysis (five independent experiments) included ANOVA I and Duncan test. The results showed that the enzymes MDH2, ME1 and G6PD of *S. cerevisiae* BY4741 exhibited an optimal of activity at 28ºC. Secondly, it was observed a significant increase in the ROS and MDA levels when temperature range from 25 to 30°C, coun tered by a significant drop at 40°C. Thus, the increase of temperature in the range from 25 to 30°C may have blocked the renewal of cytoplasmic and mitochondrial pyruvate, slowing down the carbon flux via citric acid cycle and *de novo* fatty acids biosynthesis, assisted by G6PD. The decrease of MDH2, ME1 and G6PD activities detected at 40°C may be interpreted as cell death, confirmed by the increase in the MDA levels. The exposure to TiO2-NPs triggered an increase in the MDH2 activity in any realized assays, effect that was more pronounced at 28°C. On the other hand, the ME1 acti vity which decreased in yeast grown at 25°C and 28°C, exposed to TiO 2-NPs, underwent an increase in yeasts grown at 30°C or 40°C. Although the ROS levels have incre ased with the presence of TiO2- NP in any realized assays, it was only detected an increase of cell damages in cell grown at 25, 28 and 40°C. Thus, it can be inferred that exposure of *S. cerevisiae* BY4741 to TiO2-NP can counteract the adaptation to temperature of their energy metabolism, reversing the cytoplasmic and mitochondrial pyruvate availability, that in the latter case only occurred at 30 and 40ºC.

Acknowledgement

This work is funded by FEDER Funds through the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT – Foundation for Science and Technology under the Strategic Project PEst-C/AGR/UI0115/2011.