Titanium dioxide nanoparticles exposure in heat-shock conditions reverses glucose-induced fermentation of *Saccharomyces cerevisiae*

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The consume of glucose by alcoholic fermentation in *Saccharomyces cerevisiae* involves a step of decarboxylation of pyruvate to acetaldehyde, catalysed by the enzyme pyruvate decarboxylase (PDC) that is followed by the reduction of acetaldehyde to ethanol by the enzyme alcohol dehydrogenase (ADH). In general *S. cerevisiae* uses the aerobic alcoholic fermentation to oxidize NADH in NAD^+, generated by the glycolytic pathway, when glucose is available as carbon source.

The aim of this study was: (a) to induce the aerobic alcoholic fermentation in the *S. cerevisiae* UE-ME_3 grown in YP medium with glycerol (4%) (YPG) by the addition of glucose (2%) (YPGD), and (b) to evaluate a possible modulating effect on this metabolic change by the heat shock or TiO_2-NP exposure in heat shock conditions.

Titanium dioxide nanoparticles (TiO_2-NP) with molecular size less than 100 nm were added to the culture of *S. cerevisiae* UE-ME_3 100 min after the addition of glucose, maintaining the agitation conditions (180 rpm) and the temperature at 28 ºC or still applying heat shock (28/40 ºC) (HS) by raising the temperature of the culture from 28 ºC to 40 ºC. The reading and discussion of the results included the evaluation of the influence of culture conditions on cell viability (cfu) and on the fermentative metabolism of *S. cerevisiae*, at level of the enzyme activities pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH).

The addition of glucose to the YPG medium caused an increase in the cell viability and enzyme markers of alcoholic fermentation (PDC and ADH) of *Saccharomyces cerevisiae*. After this first step it was assessed whether the heat shock (28/40ºC) or the yeast cells exposure to TiO_2-NP <100 nm (5 µg/mL) in heat shock conditions or not, for 100 min was able to reverse this effect. *S. cerevisiae* exposed to heat shock (28/40 °C) in the last 100 min of culture exhibited cell viability and level of PDC activity close to those detected in cells grown in YPG medium. However, *S. cerevisiae* in heat-shock conditions exhibited a decrease in ADH activity to the levels lower than those detected in yeasts cells grown in the control media (YPG and YPGD). The exposure of *S. cerevisiae* to TiO_2-NP <100 nm, at 100 min of growth in YPGD media caused a decrease in cell viability to levels close to those determined in yeast cells grown only in YPG medium. Interestingly, *S. cerevisiae* exposed in the last 100 min of the culture to TiO_2-NP <100 (5 µg/ml), in heat shock (28/40 °C) conditions, exhibited cell viability, PDC and ADH enzyme activities levels closed to those determined in yeast cells grown only in YPG medium. The decrease in cell viability and the slowdown of the alcoholic fermentation in aerobic conditions caused by the simultaneous exposure of *S. cerevisiae* UE-ME_3 to TiO_2-NP <100 nm (5 µg/mL) and heat-shock (28/40), after 100 min of culture in glucose, points to a negative modulation of the aerobic alcoholic fermentation in this wine wild-type yeast from Alentejo, Portugal.

Keywords: yeast; aerobic fermentation, cell viability, nanomaterials, temperature

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Transcriptomic-based analysis of the *Lactobacillus plantarum* WCFS1 response to oleuropein

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Oleuropein is the main phenolic component of olive leaves, seed, pulp and peel of unripe olive fruits and is present in higher amounts in oils obtained from green olives. This compound confers natural resistance to *Olea europeae* against both gram positive and gram negative bacteria [1]. On account of its anti-microbial activity oleuropein might play an important role to select the microbiota that colonizes the olive epidermis which is crucial for the quality of fermented table olives. Therefore it is important to increase knowledge on the oleuropein tolerance mechanisms of the olive microbiota. To this goal we have investigated how oleuropein affects the expression profile of *Lactobacillus plantarum* at genome scale since this microorganism colonizes the olive epidermis and plays an important role in the fermentation of olives [2].

Whole-transcriptome analysis was based on customized microarray profiles. Differentially expressed genes (fold-changes ≥ 2 ($p < 0.05$)) were used to perform a functional analysis by using the DAVID bioinformatics tool. The transcriptomic response revealed differential expression of genes involved in the transport and metabolism of several carbohydrates. Other set of genes whose expression was affected by the presence of oleuropein was that dedicated to the biosynthesis of fatty acids. In addition genes involved in the biosynthesis of membrane and cell wall components were also differentially transcribed respect to controls. Stress responses, including a specific oxidative stress response, were revealed by the transcriptomic datasets indicating the anti-microbial properties of this phenolic compound.

Keywords: Transcriptomics; Oleuropein

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