



**Best CIHEAM Master Thesis**

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**Effect of small antioxidant molecules on the viability of oxidative stress defective yeast**

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## Abstract

Oxidative stresses is produced in cells by oxygen derived species resulting from cellular metabolism and from interaction of cells with exogenous sources such as carcinogenic compounds, red ox-cycling drugs and ionizing radiations; H<sub>2</sub>O<sub>2</sub> and CHP are compounds that can generate free radicals. In this work we studied the capacity of seven (7) small antioxidants molecules (Ascorbic Acid, Caffeic Acid, Catechine, Beta-carotene, Quercetine, Tocotrienol ( $\beta$  and  $\gamma$ ) and Hesperidin) to protect 3 strains of yeast from oxidative stress in the presence of defined concentration of one of the two pro-oxidant H<sub>2</sub>O<sub>2</sub> or CHP. Two of the strains used have different mutations in their antioxidant machinery  $\Delta$ sod1 a strain that is deficient in super oxide dismutase enzyme and a double knockout strain  $\Delta$ oye2glr1 deficient in the same time in Old yellow enzyme and glutathione reductase , characterized by a high concentration of oxidized glutathione (GSSG), the third strain used as control was the wild type BY4741. Regarding the results we found that AA showed a big protective effect for all strains, even for the  $\Delta$ gsh1 strain which has lacking in endogenous glutathione and need an exogenous concentration of glutathione to grow. Against that, a high concentration of quercetine and beta-carotene showed an inhibitory effect on all strains which have been confirmed by measuring the level of ROS inside the cells. When quercetine has the strongest inhibitory effect, we measured the level of GSH/GSSG in the cells to see the effect of quercetine in this level and we have seen that quercetine not only increase the concentration of GSH and GSSG inside the cell but also could not help the cells to maintain the quantity of GSH when it is exposed to H<sub>2</sub>O<sub>2</sub>. Old yellow enzyme OYE2 and OYE3 has a major role in oxidative stress and the modulation of programmed cell death processes. For this we used cells that have an endogenous carboxy-terminal fusion of the green fluorescent protein (GFP) to the OYE2 or OYE3 gene and we tried the induction of genes with small 5  $\mu$ M and high concentration 50 $\mu$ M of BC (GFP fluorescence was quantified by fluorimetry). BC gave the induction of OYE2-GFP in both concentration and Quercetine lead to the super induction of OYE2-GFP. The formation of oye2p-oye3p heterodimer has previously been associated with sensitization of cells to H<sub>2</sub>O<sub>2</sub> iduced programmed cell death. GFP-YAP1 fusion strain was generated in order to examine the change of yap1 expression to quercetine supplementation by fluomitery. The results showed no change in fluorescence intensity compared to the different doses of quercetine supplemented.