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## Induction of *Saccharomyces cerevisiae* UE-ME3 proliferation by isoproturon is independent of growth stage

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The isoproturon (IPU), 3-(4-isopropylphenyl)-1,1-dimethylurea is an active component of several pesticides applied in autumn-winter crops which persist occasionally in the soil, aquifers and biological systems, at levels higher than established by directives of European Community. This phenylurea blocks photosynthesis, inhibiting chloroplasts electron chain at level of photosystem II. Therefore, the presence of IPU in the living cells can generate ROS and consequently cause slow but continuous damage to their cellular components which are increasingly described as important factors involved in the phenomenon of biological ageing and cell death. Cells exhibit defined antioxidant defences that are depleted throughout the life cycle involving the tripeptide glutathione (GSH) and antioxidant enzyme activities glutathione reductase (GR), catalase (CATT) and alkaline phosphatase (ALP). Under normal conditions, antioxidant defence mechanisms are capable of maintaining ROS at harmless levels, but prolonged exposure can eventually result in an inability to prevent cellular damages. In general, changes in the value of the GSH/GSSG ratio, GR, CAT T and ALP activities are early indicator of sensitivity to oxidative stress. On the other hand, MDA level is also used as marker of lipid peroxidation in different biological systems. The aim of this work was to determine the response of *Saccharomyces cerevisiae* UE-ME3, a wine wild-type strain belonging to the Enology laboratory collection of University of Évora, Portugal as biological model to assess IPU toxicity in eukaryotes. *S. cerevisiae* UE-ME3 ( $10^6$  cells mL<sup>-1</sup>) 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 28°C during 3 or 72h in the absence or presence of 5 and 100 µM IPU. At the end of the experiment, samples from each treatment were taken to obtain the post-12000 g supernatant, which were used for determination of GSH, GSSG and MDA contents by fluorimetric methods, and GR, CAT T and ALP enzymatic activities by spectrophotometry. Statistical analysis was performed by ANOVA one-way and Duncan-test. The results showed that *S. cerevisiae* grown until lag phase, mitotically more active, have showed higher values in ALP activity, non-protein thiols, glutathione disulfide and MDA than yeast cells grown until stationary phase. Conversely, an inverse relationship was observed for the enzyme activities GR and CAT T as well as GSH levels and GSH/GSSG ratio. Thus, fermentative cells exhibited lower effectiveness in stabilizing the reducing environment mediated by antioxidant enzymes, fact that may be related with an increase for occurrence of cell damages. On the other hand, the IPU exposure caused a significant increase in the enzyme activities ALP, CAT T and GR without affect cell reducing environment in both growth periods and exposition levels. These results suggest that the IPU caused an increase of cell proliferation, assisted in part, by CAT T activity and glutathione cycle which have been more effective in *S. cerevisiae* exposed to IPU until stationary phase/respiratory, which may have determined a significant decrease in the levels of MDA. However, an opposite effect was detected in *S. cerevisiae* exposed to IPU until lag phase, response that pointed toward a significant increase of oxidative damages due a slow effectiveness antioxidant response of these cells.

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