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# XI CONGRESSO NACIONAL DE BIOQUÍMICA

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## PROGRAMA e RESUMOS

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staining protocol with a highly specific DNA dye for the analysis of the yeast cell cycle (CC) was developed. With this new protocol, it was possible to achieve a considerable improvement in the variation coefficients of the G0/G1 and G2/M peaks and a clear cut identification of the S phase. Furthermore, this apparent improvement allowed a more rigorous assessment of the effects of benomyl and diclofluanid in the CC of the yeast, when compared with the staining protocol with propidium iodide. Changes in the cellular DNA content induced by the fungicides could also be estimated by the introduction of an internal standard in the samples for cell cycle analysis. CC analysis of yeast samples incubated with benomyl revealed a clear accumulation of cells in G2/M, proportional to the concentration of the fungicide. On the other hand, incubation with diclofluanid seems to have little or no effects on the relative distribution of cells in the CC. However, we were able to detect slight changes in the cellular DNA content induced by the the presence of this fungicide.

\* Margarida Fortuna received a fellowship from INIA-PAMAF nº 97.09.5520.9 (Portugal).

P3 - 15

ANSWER FROM THE YEASTS *ZYGOSACCHAROMYCES BAILII* AND *SACCHAROMYCES CEREVISIAE* TO THE PRESENCE OF FUNGICIDES - BENOMYL AND CIMOXAMIL

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Sometimes it was detected residues of fungicides in must. These products cause a small inhibitory effect on fermentation. Nevertheless, when applied 8 a 15 days before the harvest, it could affect negatively the fermentation.

In the present work the effects of benomyl and cimoxamil in growth and cell viability of *Zygosaccharomyces bailii* ISA 1307 and of a *Saccharomyces cerevisiae* isolated from a grape must at the Adega Cooperativa de Murça, were studied. At pH 4.0 and 26 °C, the presence of benomyl and cimoxamil, above a minimum inhibitory concentration (MIC) decreased the specific growth rate and the final biomass of both species, the reduction increasing with the concentration of the fungicide in the extra-cellular medium. For both species and the two fungicides, when present at concentrations above the MIC value, the inhibition kinetics was expressed by an exponential relation. The dependence of the observed effects in growth and final biomass of both species although qualitatively identical, was quantitatively different. Actually, the value of the MIC of benomyl in *Z. bailii* ISA 1307 was lower than in *Saccharomyces cerevisiae*. In turn, the values of the exponential inhibition constants of growth for benomyl and cimoxamil were higher in *Z. bailii* ISA 1307.

In what concerns the cell viability, the effects of the two fungicides on the specific death rates were studied under isothermal conditions (25 °C) and pH 3.0. For both species and both fungicides, cellular death was exponentially stimulated in the presence of increasing extra-cellular fungicide concentrations. The exponential enhancement constants of cellular death by benomyl and cimoxamil in *Z. bailii* ISA 1307 were lower than in *Saccharomyces cerevisiae*. The results suggested that *Z. bailii* ISA 1307, comparatively to *Saccharomyces cerevisiae*, is more resistant to benomyl and cimoxamil, at least in what regards cell viability.

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P3 - 18

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