

Implemented Activities

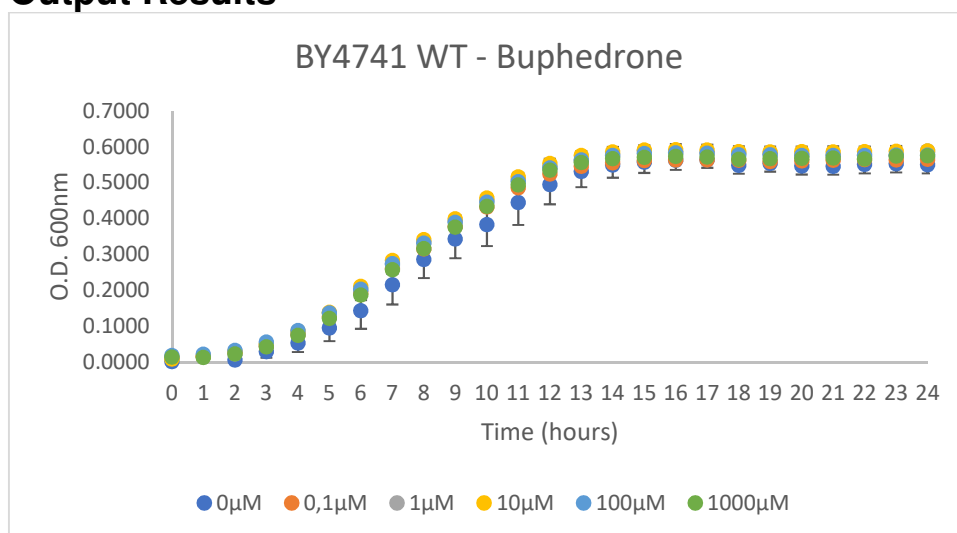
Yeast growth kinetics was implemented to find the best NPS candidates for toxicological assays with human cells and to find the range of concentration to be used on those assays. Therefore, growth kinetics with 3 strains of *Saccharomyces cerevisiae* was performed. The strains studied were BY4741 WT and two mutant strains: Δ PDR5 e AD1 \rightarrow 8. *Saccharomyces cerevisiae* mutants' strains have mutations in ABC pumps, which are involved in the efflux of the most xenobiotics. The main purpose to use these strains is to try to reduce the concentration of NPS used in yeast cells.

The assay was developed using buphedrone. The first approach, where sub-mM range of buphedrone was used, was ineffective. The kinetic growth was identical for the different concentrations. In addition the mutants used had identical growth curves when compared to the WT strain. Therefore, mutant strains were abandoned and WT strain was used to determine which concentrations should have been tested in human cell lines. Buphedrone concentration was increased till 100 mM till we get an observed effect on yeast growth. The results with *Saccharomyces cerevisiae* WT strain suggests that the concentration range of buphedrone to be used in human cells should be between 0mM and 10mM.

Activities Delayed

At this point, there were more substances that should be under analysis. This was not possible yet due to the lack of a final list and some difficulties to buy the standards. Moreover the proteomics assays have not started yet due to the lack of substances.

Output Results



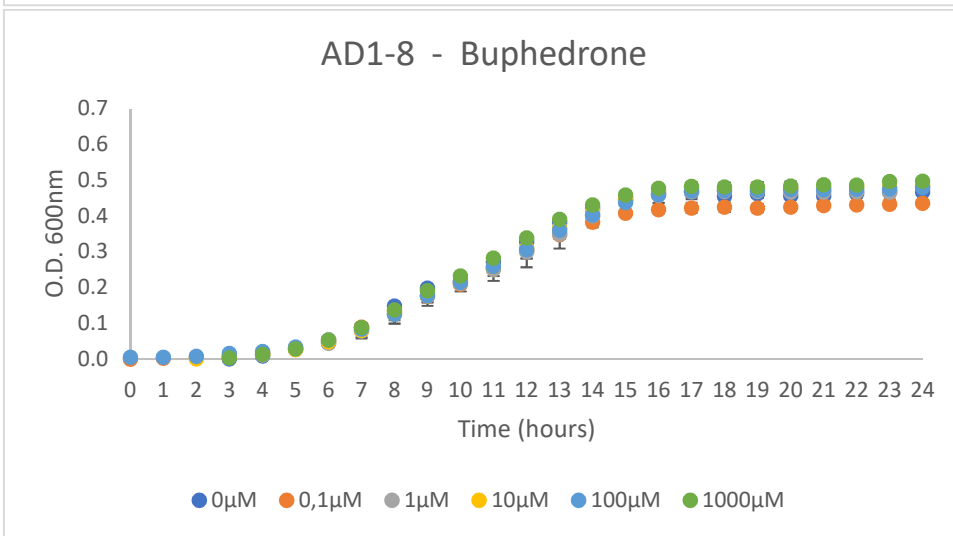
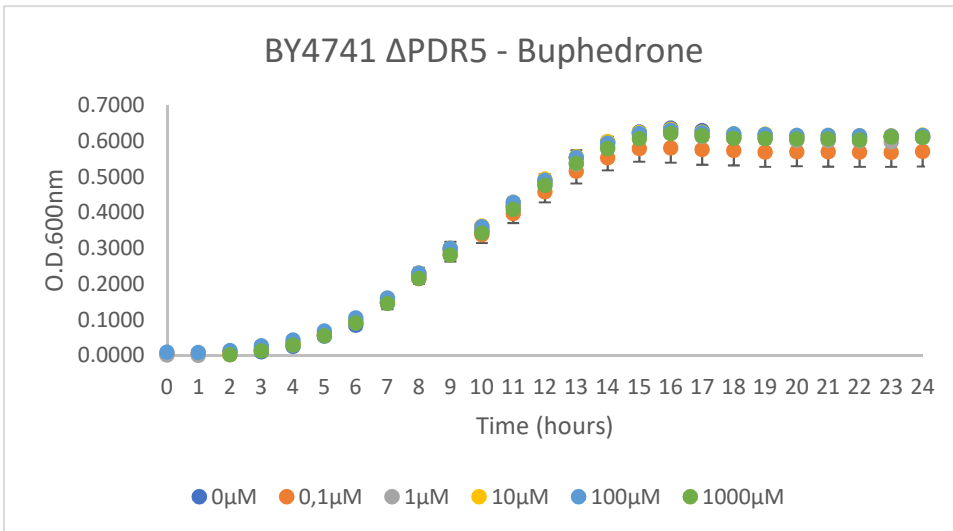


Fig. 1 – Kinetic growth of 3 different strains of *Saccharomuces cerevisiae* (BY4741 WT; BY4741 Δ PDR5; and AD1 \rightarrow 8) in the presence of 0 μ M; 0,1 μ M; 1 μ M; 10 μ M; 10² μ M and 10³ μ M of Buphedrone.

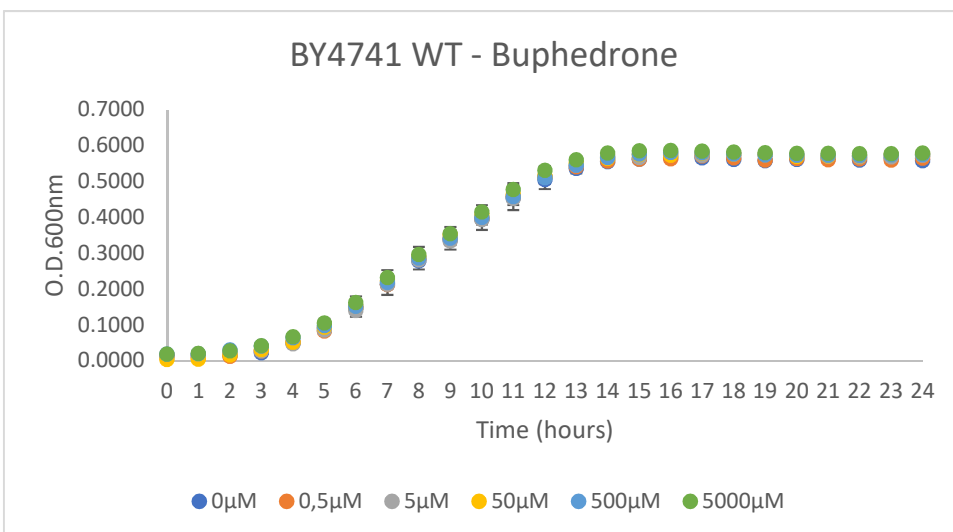


Fig. 3 – Kinetic Growth of *Saccharomyces cerevisiae* BY4741 WT in the presence of 0 μM ; 10 μM ; 10² μM ; 10³ μM ; 10⁴ μM and 10⁵ μM of Buphedrone.

Deliverable Results

According to the results and known that yeast cells needed approximately 100x more concentration of drugs when compared to human cells, we had suggested to the group of FFUL, who is testing NPS in human cell lines, to use a concentration range of buphedrone till 10 mM.