Analysis of DNA damage and repair in *Saccharomyces cerevisiae* using the comet assay in the characterization of antigenotoxicity of plant extracts and phytochemicals

Flávio Azevedo[1], Filipe Marques[1], Hanna Fokt[1], Alberta Domingues[1,2], Alberto Dias[2], Björn Johansson[1] and **Rui Oliveira**[1]*

1 CBMA (Molecular and Environmental Research Center)/Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

2 CITAB (Centre for the Research and Technology of Agro-Environmental and Biological Sciences), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga Portugal

* ruipso@bio.uminho.pt

Objectives

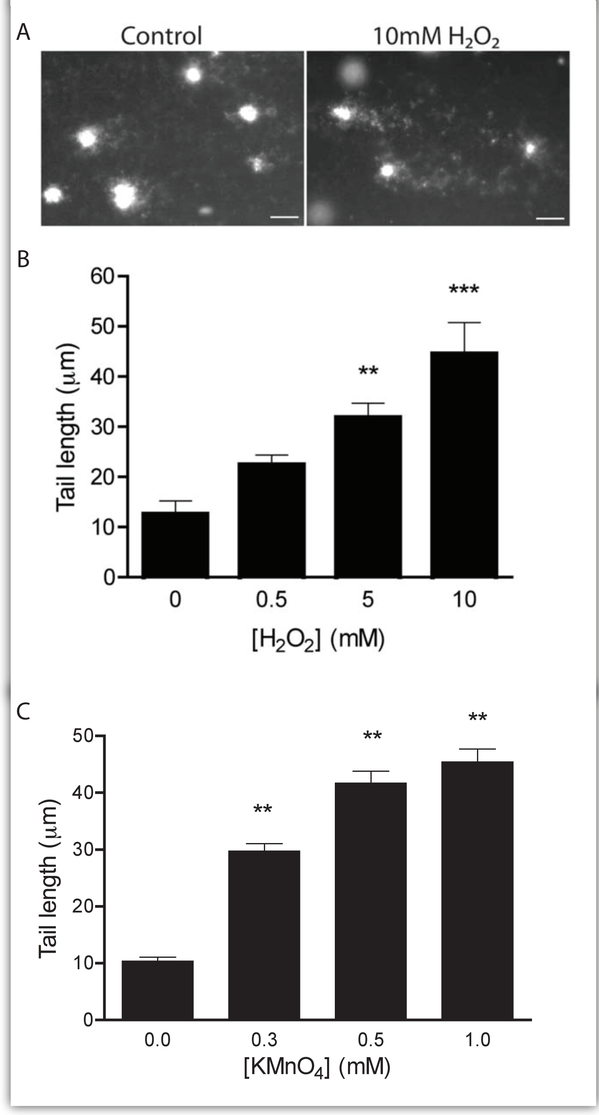
Explore yeast as experimental model in the characterization of antigenotoxicity of phytochemicals

Characterize antigenotoxicity of *Ginkgo biloba* leaf extracts and its phytochemicals

Explore yeast genetic tools and mutants to study mechanisms of antigenotoxicity of *G. biloba* phytochemicals

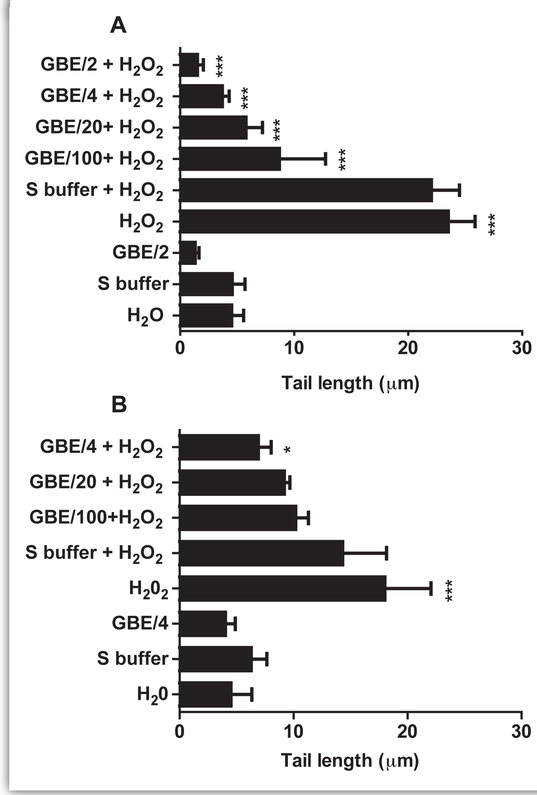
DNA damage of yeast cells is dependent on the concentration of H_2O_2 and $KMnO_4$

(A) Image samples obtained by the application of the alkaline version of the comet assay in untreated (control) and treated (10mM H_2O_2) yeast cells. Bar=10µm.
(B, C) DNA damage as represented as mean ±SD tail length of three independent experiments with at least 50 comets scored per experiment for each concentration of H_2O_2 (B) or $KMnO_4$ (C). **p<0.01 and ***p<0.001.



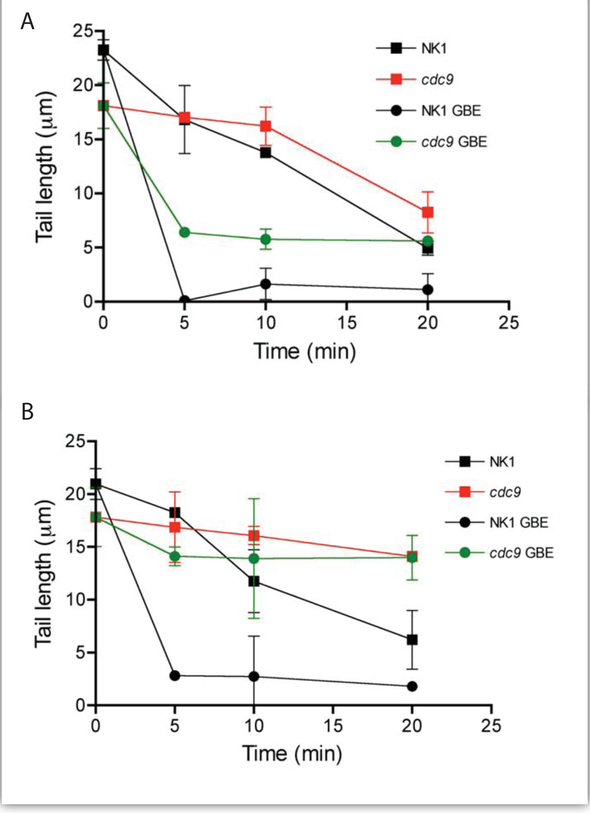
Ginkgo biloba leaf extract (GBE) protects yeast cells from oxidative damage by H_2O_2

Yeast spheroplasts were incubated with GBE (diluted 2, 4, 20 and 100-fold in S buffer), for 20min, washed with S buffer, and incubated with 10mM H_2O_2 for 20min (A) or incubated simultaneously with both for 20min (B). DNA damage was analyzed with the alkaline comet assay. "S buffer+H_2O_2" and "H_2O_2" denote positive controls and "GBE/2", "GBE/4", "S buffer" and "H_2O" denote negative controls. Mean ±SD values are from at least three independent experiments. *p<0.05, **p<0.01 and ***p<0.001.



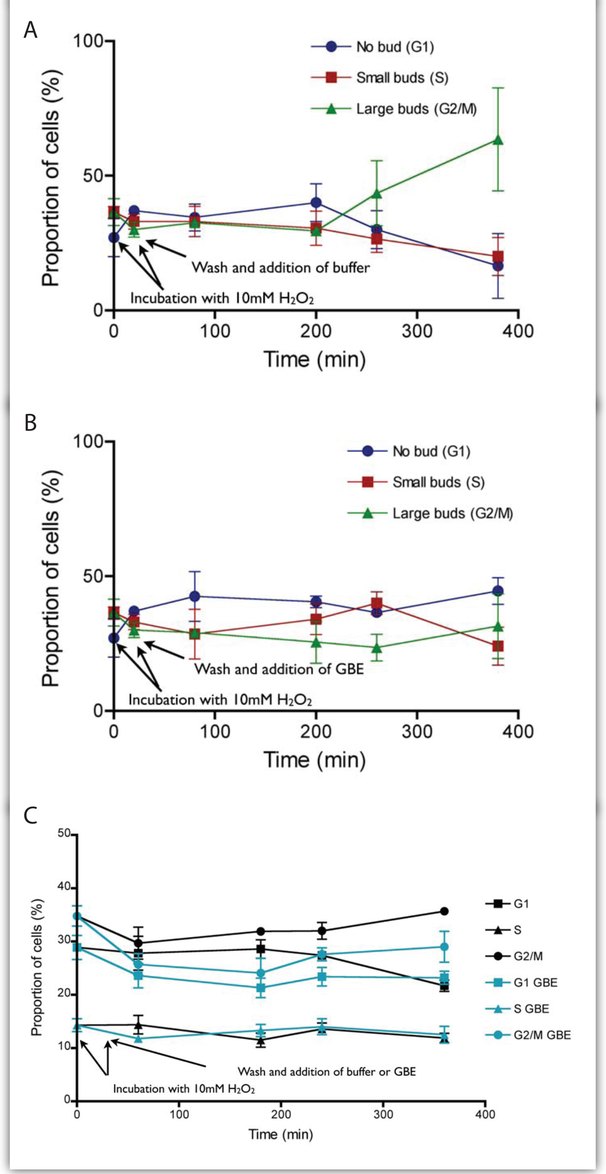
Ginkgo biloba leaf extract (GBE) increases DNA repair ability in yeast cells upon damage by H_2O_2. GBE does not improve DNA repair in the DNA repair-defective mutant (NK427, *cdc9*)

(A) Spheroplasts of yeast parental strain NK1 (black) and *cdc9* temperature-sensitive mutant NK427 (red and green) were incubated with 10mM H_2O_2 for 20min at the permissive temperature of 23ºC, washed with S buffer and incubated with GBE (circles) or S buffer (squares) at 23ºC. At each time-point spheroplasts were washed with S buffer and DNA damage was analyzed with the alkaline comet assay. (B) The same as A except for the additional 1h incubation of cells at the restrictive temperature of 37ºC before the experiment and all subsequent incubations at 37ºC instead of 23ºC. All results are the mean of three independent experiments.



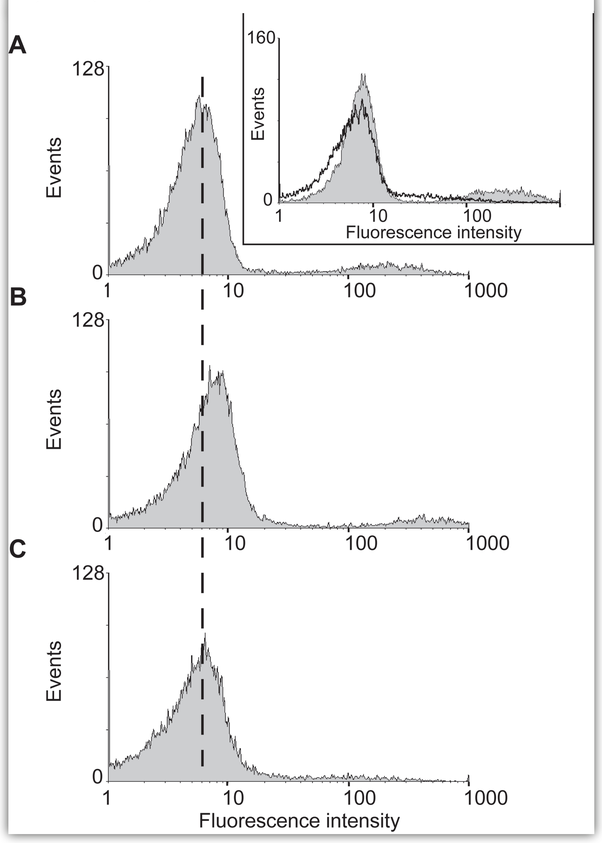
GBE suppresses cell cycle arrest of yeast cells in G2/M caused by H_2O_2

Yeast cells were incubated with 10mM H_2O_2, washed and ressuspended in buffer (A) or GBE (B). At each time-point, an aliquot was harvested and cell cycle was analyzed by determination of the budding index of cells (no bud=G1; small bud=S; and large bud=G2/M). (C) The same as A and B except for the analysis of cell cycle by flow cytometry using SYBR green for DNA quantification. Results are the mean of three independent exeriments.



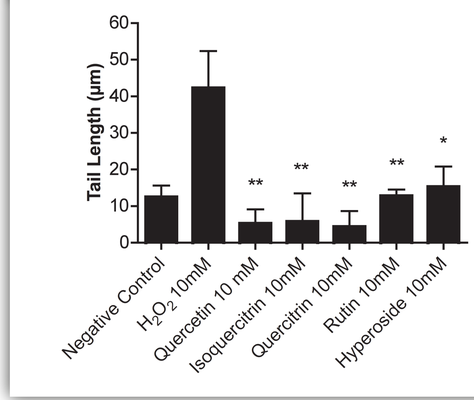
GBE decreases intracellular oxidation in yeast cells

Yeast cells were loaded with the redox-sensitive fluorochrome dichlorofluorescein diacetate (H_2DCFDA) for 60min in the dark. After washing with buffer, cells were analyzed by flow cytometry for fluorescence of the oxidized form of the fluorochrome (DCF) (A); or incubated with 10mM H_2O_2 for 20min, washed with buffer and analyzed by flow cytometry (B); or incubated with GBE for 20min, washed with buffer, incubated with 10mM H_2O_2, washed with buffer and analyzed by flow cytometry (C).Inset: the same as before except for incubation with only GBE unshaded or only buffer (shaded) for 20min.



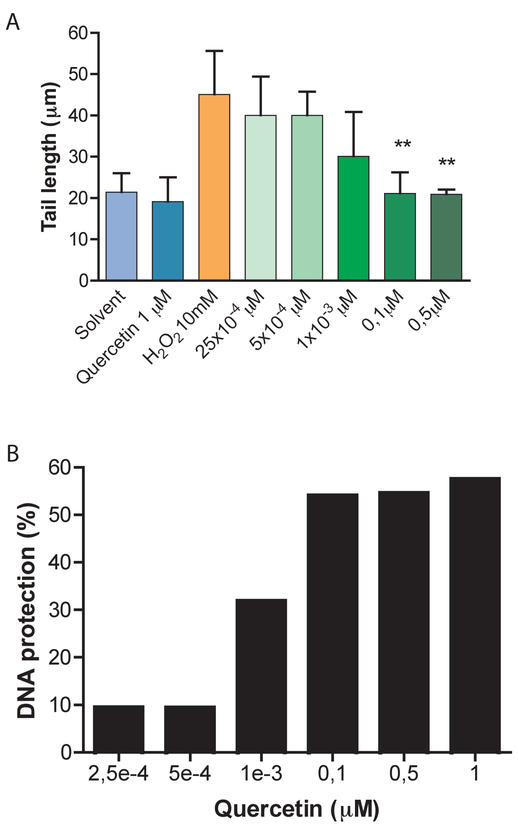
Plant flavonoids protect yeast cells from oxidative damage by H_2O_2

Yeast spheroplasts were incubated with plant flavonoids for 20min, washed with S buffer, and incubated with 10mM H_2O_2 for 20min. DNA damage was analyzed with the alkaline comet assay. "H_2O_2 10mM" denotes positive control and negative control was done with S buffer. Mean ±SD values are from at least three independent experiments. *p<0.05 and **p<0.01.



Pre-incubation with low concentrations of quercetin is sufficient for DNA protection from oxidative damage by H_2O_2 in yeast cells

(A) Yeast spheroplasts were incubated with different concentrations of quercetin for 20min, washed with S buffer, incubated with 10mM H_2O_2 for 20min and DNA damage was analyzed with the alkaline comet assay. "H_2O_2 10mM" denotes positive control and "Solvent" and "Quercetin 1µM" denote negative controls. Mean ±SD values are from at least three independent experiments. **p<0.01.
(B) Taking the value of the positive control as reference (0% protection), protection was calculated as the percentage of decrease upon pre-treatment with each compound.



Conclusions

Saccharomyces cerevisiae can be used as experimental model in genotoxicity and antigenotoxicity assays

Conditional mutant yeast strains affected in essential genes (like *CDC9* encoding a DNA ligase involved in NER and BER) are useful to explore the mechanism of action of phytochemicals

Antigenotoxic activity of *Ginkgo biloba* leaf extract can be mediated by its antioxidant activity and by its capacity of improvement of DNA repair kinetics, presumably by inducing NER and/or BER

Ginkgo biloba leaf extract suppresses hydrogen peroxide-induced cell cycle arrest at G2/M

Common plant flavonoids, including quercetin found in *G. biloba* extracts and quercitrin and its glycosides (isoquercitrin, rutin and hyperoside) are antigenotoxic

Acknowledgements

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References

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