THE *Ashbya gossypii* COMET ASSAY: MEASURING OXIDATIVE AND NON-OXIDATIVE DAMAGE IN THE DNA

CENTRE OF BIOLOGICAL ENGINEERING

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INTRODUCTION

- The Comet Assay (Single Cell Gel Electrophoresis) is one of the most used techniques for measuring DNA damage[1] due to its simplicity, sensitivity and versatility[2].
- The theoretical scientific basis of this methodology consists on the increased electrophoretic mobility of damaged DNA.
- It has a broad range of applications such as: compounds' genotoxicity/protective testing, DNA repair studies and ecological monitoring.
- With a huge amount of optimized protocols for superior eukaryotes, the Comet Assay has been poorly utilized among microorganisms.
- However, Azevedo et al. (2011)[3] developed an optimized protocol for *Saccharomyces cerevisiae* that reinforce the utility and potential of this method for microbial systems.
- **Therefore, in this work we created a reproducible and optimized Comet Assay protocol for use in *Ashbya gossypii* by adaptation of the Yeast Comet Assay[3] (Fig. 1). This protocol allows the measuring of oxidative and non-oxidative DNA damage.**
- **Moreover, as riboflavin overproduction in *A. gossypii* has been associated with stress (mainly oxidative), we went to check if this trait was also associated with increased DNA damage. Therefore, we used this recently created protocol to investigate the DNA damage accumulation in riboflavin overproducing and non-overproducing *A. gossypii* wild strains.**

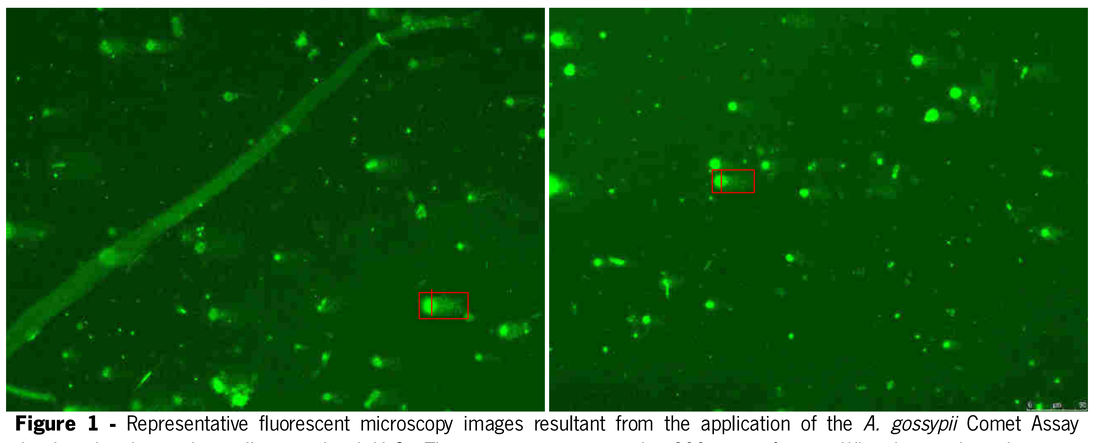
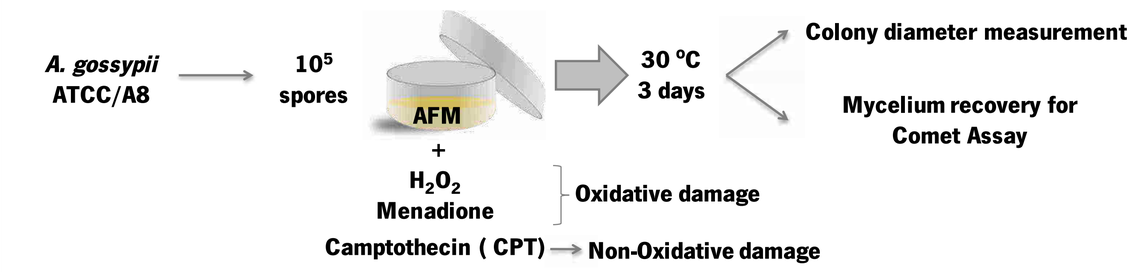


Figure 1 - Representative fluorescent microscopy images resultant from the application of the *A. gossypii* Comet Assay developed in this work in cells treated with H_2O_2. The images were acquired at 200x magnification. White bar on the right image = 50 µm.

METHODS

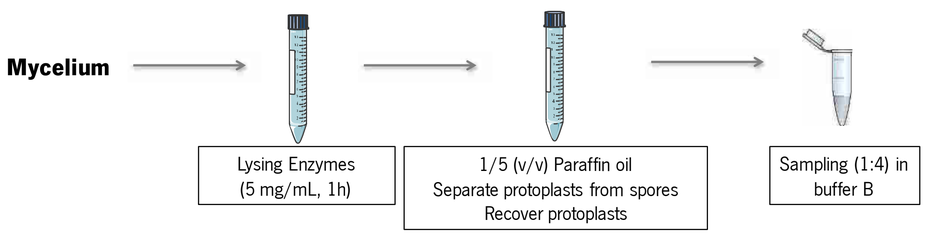
1. RADIAL GROWTH OF *A. gossypii* WILD STRAINS



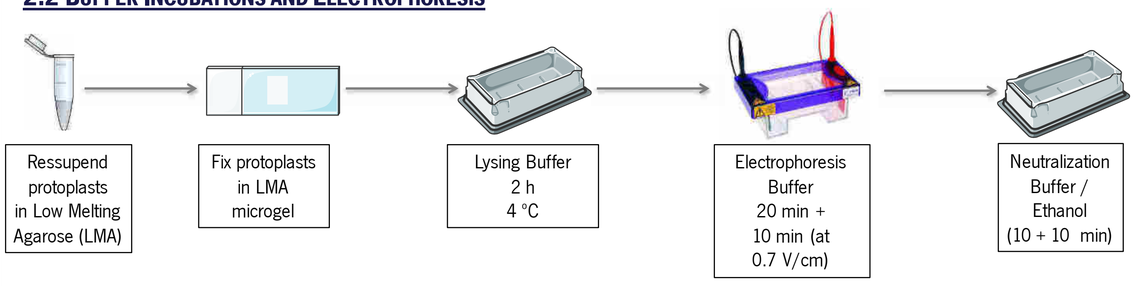
- *A. gossypii* ATCC10895 (kindly provided by Prof. P. Philippsen, University of Basel) and *A. gossypii* A8 (kindly provided by Prof. J.L. Revuelta, University of Salamanca) .
- For growth under light exposure a fluorescent lamp (LUMILUX T5 HE, OSRAM) was used with an irradiance level of 16 µmol m−2 s−1, measured by a LI-250 Light Meter with a LI-190 quantum sensor (LI-COR, USA).

2. THE *A. gossypii* COMET ASSAY

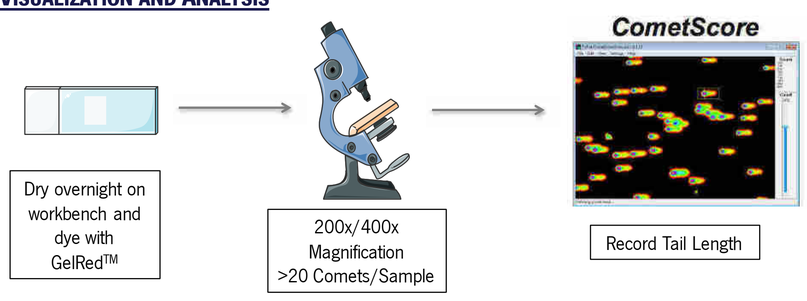
2.1 PROTOPLAST GENERATION



2.2 BUFFER INCUBATIONS AND ELECTROPHORESIS

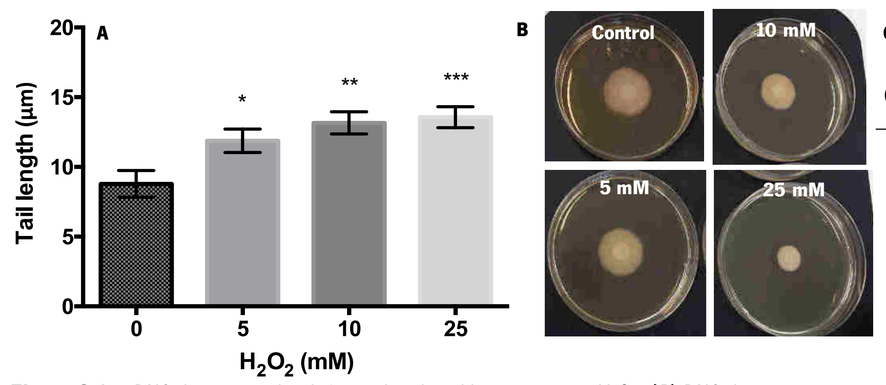


2.3 COMETS VISUALIZATION AND ANALYSIS



RESULTS

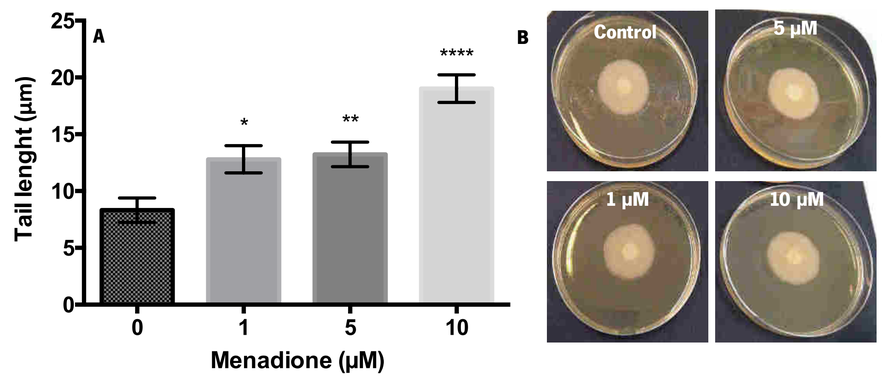
1. THE *A. gossypii* COMET ASSAY IS ABLE TO MEASURE OXIDATIVE (H_2O_2 AND MENADIONE) AND NON-OXIDATIVE (CPT) DNA DAMAGE



C

Condition [H_2O_2]	Relative Radial Growth (%)
5 mM	92
10 mM	79
25 mM	51

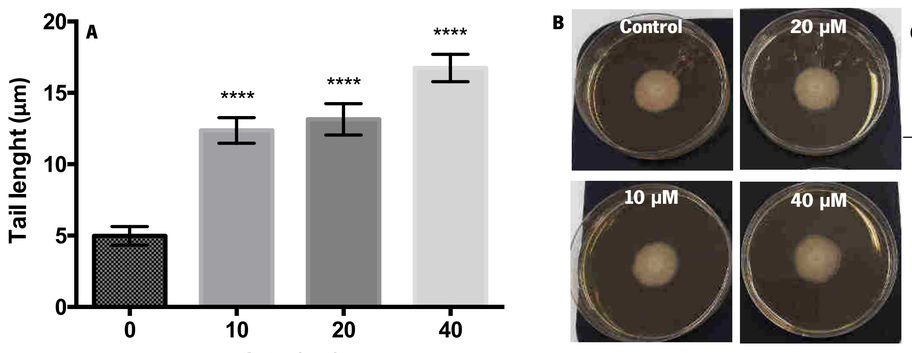
Figure 2.1 – DNA damage and radial growth induced by exposure to H_2O_2. **(A)** DNA damage is represented as mean ± SEM of the tail length. **(B)** Representative photos of mycelia after growth for 3 days exposed to different concentrations of H_2O_2. **(C)** Relative growth of cells to H_2O_2 in comparison to the control condition (0 mM H_2O_2).



C

Condition [Menadione]	Relative Radial Growth (%)
1 µM	100
5 µM	98
10 µM	100

Figure 2.2 – DNA damage and radial growth induced by exposure to Menadione. **(A)** DNA damage is represented as mean ± SEM of the tail length. **(B)** Representative photos of mycelia after growth for 3 days exposed to different concentrations of Menadione. **(C)** Relative growth of cells exposed to Menadione in comparison to the control condition (0 µM Menadione).



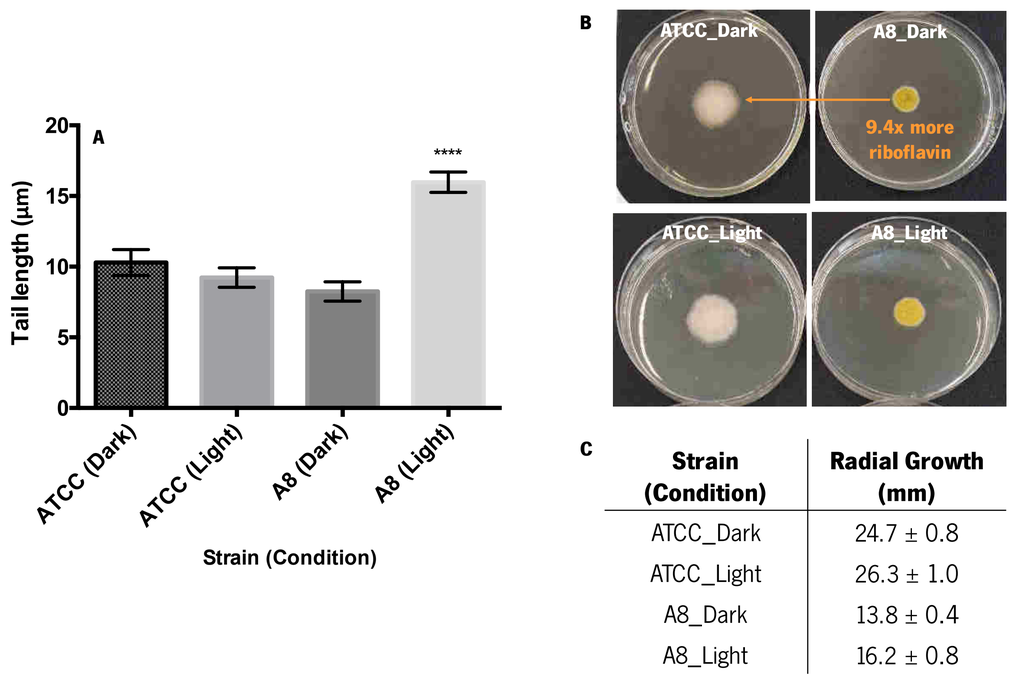
C

Condition [CPT]	Relative Radial Growth (%)
10 µM	96
20 µM	99
40 µM	100

Figure 2.3 – DNA damage and radial growth induced by exposure to CPT. **(A)** DNA damage is represented as mean ± SEM of the tail length. **(B)** Representative photos of mycelia after growth for 3 days exposed to different concentrations of CPT. **(C)** Relative growth of cells exposed to CPT in comparison to the control condition (0 µM CPT).

Statistic tests were determined by one-way ANOVA and Sidak's multiple comparisons test ($p < 0.5$). (*) represent statistically significant differences in comparison with the control condition (0). 3 biological replicates for each condition.

2. RIBOFLAVIN OVERPRODUCING STRAIN (A8) ONLY ACCUMULATES MORE DNA DAMAGE WHEN EXPOSED TO LIGHT DURING GROWTH



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Strain (Condition)	Radial Growth (mm)
ATCC_Dark	24.7 ± 0.8
ATCC_Light	26.3 ± 1.0
A8_Dark	13.8 ± 0.4
A8_Light	16.2 ± 0.8

Figure 3 – DNA damage and radial growth of different strains not exposed/exposed to light during the growth. **(A)** DNA damage is represented as mean ± SEM of the tail length. **(B)** Representative photos of mycelia after growth for 3 days in the different conditions tested. **(C)** Radial growth of mycelia in the different conditions tested (data are represented as mean ± SD).

CONCLUSIONS

- We efficiently **adapted** the **Yeast Comet Assay for use in *A. gossypii*** with some alterations to the original protocol.
- This adapted protocol is a suitable tool to **measure oxidative** and **non-oxidative damage** in the **DNA** of ***A. gossypii*,** demonstrating **high sensitivity** and **response to different stress agents at different concentrations**.
- Using this method we report that **a riboflavin overproducing *A. gossypii* wild strain (A8)** accumulates **more DNA damage** than a non-overproducing strain when it is **exposed** to **light**, suggesting a **photosensitizer** role of **riboflavin** in these conditions, as already observed in mammalian cells[4,5].

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