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Challenges of the comet assay on yeast cells

Jivulescu Laura-Elena[1,2], Menghiu Gheorghita[1,2*]

[1] Advanced Environmental Research Laboratories; West University of Timisoara, Oituz 4A, 300086 Timisoara, Romania,
[2] Department of Biology–Chemistry; Faculty of Chemistry, Biology, Geography, West University of Timisoara, Pestalozzi 16, Timisoara 300115, Romania
*Corresponding author e-mail: gheorghita.menghiu@e-uvt.ro

Abstract: Yeast cells are eukaryotic cells, which are easy to grow, inexpensive and do not require much time to study. The main challenge of using yeast in the comet assay is the necessity to degrade the cell wall, which is why they are poorly studied. In this work, the response of *Pichia pastoris* and *Saccharomyces cerevisiae* cells to the comet assay was investigated.

Introduction

Comet assay or single cell gel electrophoresis is a sensitive, reliable and rapid method for the detection of double and single stranded DNA breaks, DNA alkaline labile sites and delayed repair sites in individual eukaryotic cells. Applications of the comet assay are targeted towards DNA repair studies, genotoxicology, clinical field, environmental biomonitoring and human monitoring.

Material and method

The yeast culture was initiated 48 hours prior to experimentation, with agitation maintained at 250 rotations per minute under ambient conditions.

Centrifugation of tubes and resuspension of resulting cells in 10 µL distilled water.

Pre-coating of five microscope slides with 1% agarose LMP.

Mixing 5 µL cellular suspension with 5 µL agarose LMP for each strain, dispensing two droplets (10 µL each) onto every slide for *S. cerevisiae* and *P. pastoris.* Slides underwent various treatments (Fig.2).

Lysis with NaCl, EDTA and Tris solution for 1 hour, then enzyme solution applied and incubated at 37°C for 30 minutes.

Introduction of treated slides into electrophoresis chamber with 0.3 M NaOH and 1 mM EDTA solution.

Neutralization in 0.4 M Tris buffer for 10 minutes, staining with ethidium bromide, and analytical examination.

Fig.1. Schematic representation of comet assay working steps

Results and discussions

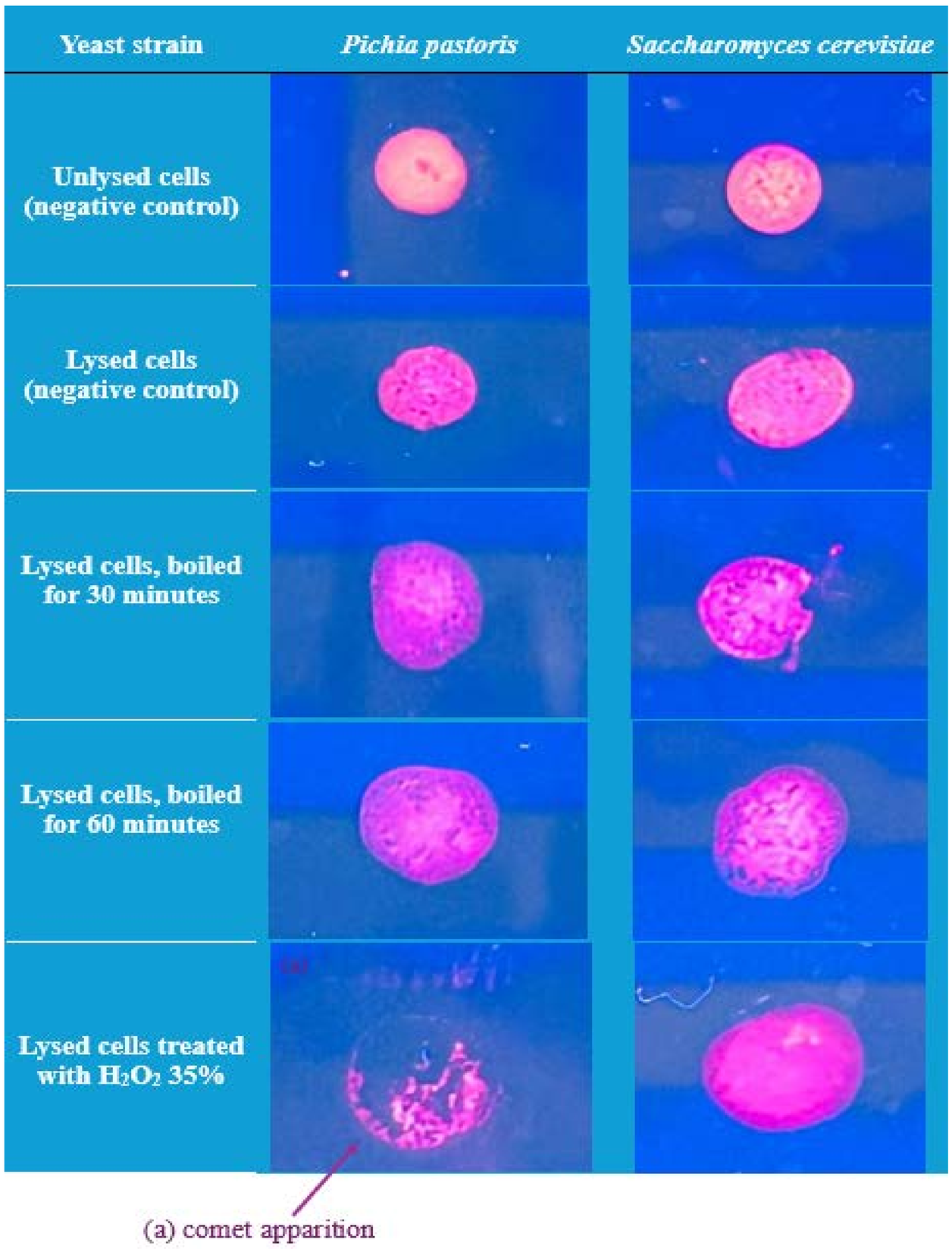

Fig. 2. Comparative analysis of single cell cultures responses of *Pichia pastoris* and *Saccharomyces cerevisiae* under various experimental conditions with observation of comet tail formation (a)

Conclusions

Results showed that the cell cultures treated for 1 h with 35% hydrogen peroxide solution exhibits an observable comet tail in Pichia pastoris. The amount of DNA in one yeast cell is low, and the single cell research remains under optimization and attention, regarding comet assay. However, when single cell cultures were performed and optimized the denatured DNA was visible without microscope.

References:
COLLINS, Andrew, et al. Measuring DNA modifications with the comet assay: a compendium of protocols. *Nature protocols*, 2023, 18.3: 929-989. DOI: 10.1038/s41596-022-00754-y. PMID: 36707722.
RANK, Jette; SYBERG, Kristian; JENSEN, Klara. Comet assay on tetraploid yeast cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 2009, 673.1: 53-58. DOI: 10.1016/j.mrgentox.2008.11.014. PMID: 19146983.

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