Molecular Microbiology and Microbial Physiology

O-25 - ASHYBA GOSSYPII RIBOFLAVIN OVERPRODUCING STRAINS ARE HIGHLY SUSCEPTIBLE TO LIGHT-INDUCED OXIDATIVE DNA DAMAGE

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Background
The overproduction of riboflavin by Ashbya gossypii, which is one of the most distinctive traits of this filamentous hemiascomycete, is triggered by oxidative stress [1]. In turn, riboflavin is a strong photosensitizer that upon irradiation with light has been shown to generate reactive oxygen species (ROS) and induce oxidative DNA damage in mammalian cells [2-3]. Envisioning a better understanding of this A. gossypii trait, here we investigated whether riboflavin overproduction is associated with increased DNA damage.

Method
The DNA damage accumulation in riboflavin overproducing and non-overproducing A. gossypii wild strains was assessed with a newly developed Ashbya Comet Assay (Single Cell Alkaline Gel Electrophoresis). This protocol is an adapted and optimized version of the Yeast Comet Assay [4] and is here shown to reproducibly measure oxidative (H2O2/menadione-mediated) and non-oxidative (camptothecin-mediated) DNA damage in A. gossypii. Radial growth and riboflavin production was assessed on agar-solidified AFM after incubation in the dark or under a visible fluorescent lamp for three days.

Results & Conclusions
The newly developed Ashbya Comet Assay allowed the reproducible measurement of H2O2/menadione-mediated (oxidative) and camptothecin-mediated (non-oxidative) DNA damage. Further assessment of the DNA damage in different A. gossypii wild strains with this validated protocol revealed significantly higher DNA damage accumulation in the riboflavin overproducing strain when it was exposed to light during growth. However, no significant differences were observed in terms of growth or riboflavin production by this strain. The non-overproducing strain did not display significant differences between conditions in any of the measured parameters. These evidences show that the accumulation of riboflavin in A. gossypii makes it highly susceptible to light-induced oxidative DNA damage, similarly to what occurs in mammalian cells [2-3]. These results thus draw attention for the importance of controlling the exposure to light of biotechnological riboflavin production processes (with A. gossypii or other organisms).

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References

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