Assessing the role of dityrosine in the UV protection of yeast spores at varying wavelengths

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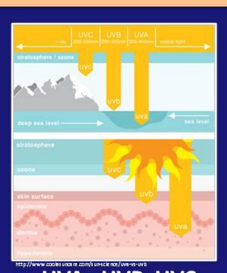
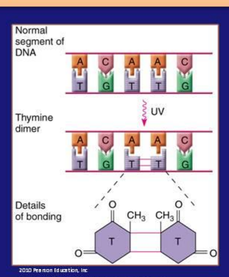
Abstract

Yeast spores are known to be resistant to many environmental factors including the mutagenic effects of UV light. The presence of dityrosine in their outermost spore wall may play a protective role by absorbing UV light. The DIT1 gene encodes a formyl transferase enzyme required for dityrosine production. The spores of a heterozygous DIT 1 strain of *Saccharomyces cerevisiae* were exposed to UV light at different time intervals and wavelengths and their ability to survive was examined. Spores present within tetrads (ascus) and free spores produced by glusulase digestion were analyzed. This study compared the UV sensitivity at the mutagenic wavelength, 254 nm and at 302 nm which is within the range of the absorption spectrum for dityrosine. Tetrads exposed to UV radiation at 254 nm were more susceptible than tetrads exposed at 302 nm. Only 3% survival was observed after a 90 sec UV exposure at 254 nm compared to 63% survival at 302 nm. A similar trend was observed for free spores. Only 0.5% survival was observed after a 90 sec UV exposure at 254 nm compared to 46% survival at 302 nm. The results suggest that dityrosine is most protective at 302 nm and tetrads may contribute to UV protection by allowing spores to hide within the ascus.

UV Radiation and Pyrimidine Dimers



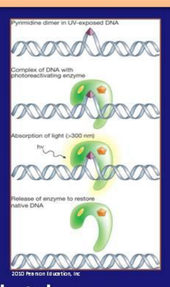
- UVA, UVB, UVC



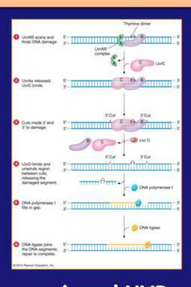
- T^T, C^C, C^T
- Replication errors

Figure 1 - Sunlight is made up of three types of ultraviolet radiation: UVC, UVA, and UVB. UVA radiation has the longest wavelengths and can penetrate deep into the dermis causing cancer and premature aging. UVB rays have intermediate wavelengths and penetrate into the epidermis casing sunburn. When two neighboring pyrimidine bases absorb UV light, they become covalently bonded via a pyrimidine dimer. This dimer may affect DNA replication if it is not properly repaired.

Repair Mechanisms



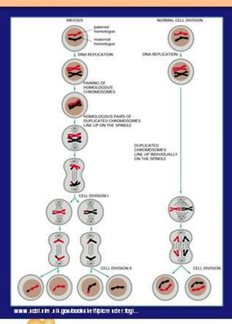
Photolyase enzyme



Excision repair and UVR enzymes

Figure 2 - Yeast cells have two repair mechanisms, photolyase and excision repair. Only the latter is found in mammals.

Life cycle of *Saccharomyces cerevisiae*
Mitosis and Meiosis



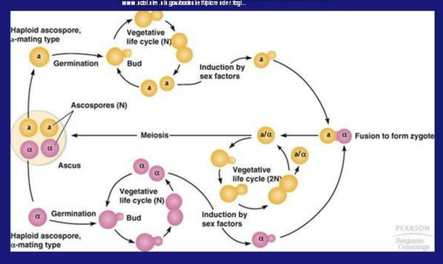


Figure 3 - In mitosis, a diploid cell divides to produce two diploid cells. In meiosis, a diploid cell divides to produce four haploid cells. Yeast exists in two forms: haploid and diploid. The haploid has two mating types, a and α and divide via budding. Opposite mating types can fuse to form a diploid cell a/α that divides via budding. Yeast cells divide mitotically in the presence of glucose but the diploid cells enter meiosis in response to starvation. Starving cells will complete Meiosis I and II and form 4 haploid spores contained within an ascus.

Dityrosine and UV wavelengths

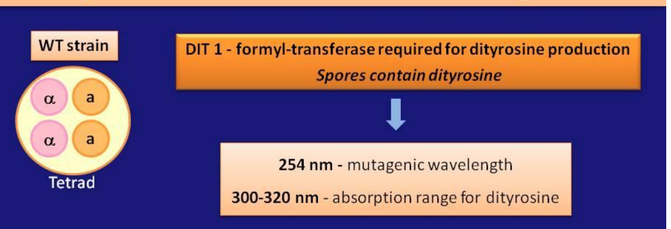


Figure 4 – Dityrosine is present in the outer spore wall. The DIT1 gene encodes for a formyl transferase required for dityrosine production. In this experiment, we assessed the protective role of dityrosine at 2 different wavelengths, 254 nm and 302 nm. The mutagenic wavelength for most cells is 254 nm whereas 302 nm falls within the dityrosine absorption range.

The Sporulation Assay

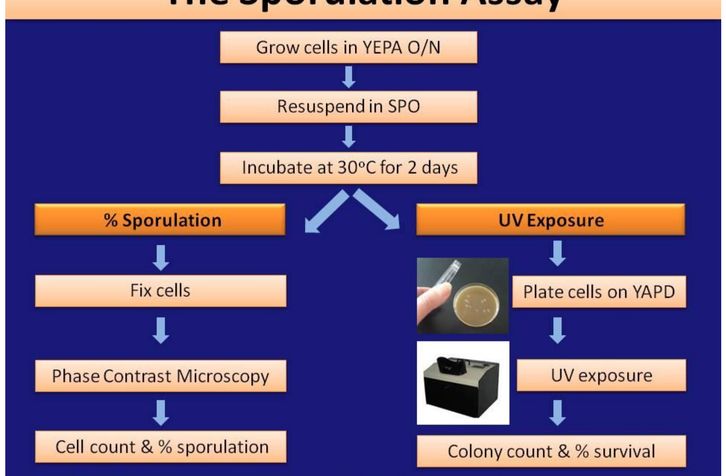
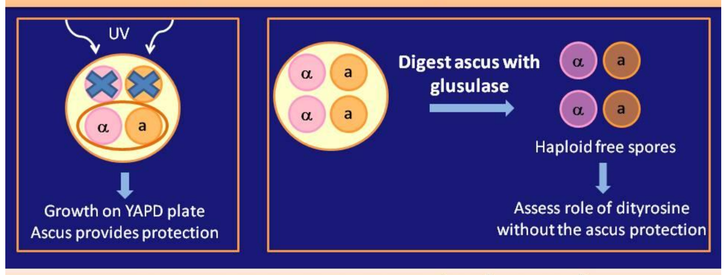


Figure 5 – Cells were grown overnight in YEPA (pre-sporulation media) and then were resuspended in SPO media (2% potassium acetate) to induce sporulation. The cells were fixed and viewed under phase contrast microscopy and the percent sporulation was calculated. The spores were then plated on YAPD plates (glucose containing media) using roller beads to ensure even distribution. The plates were then exposed to UV light for different time intervals and were incubated at 30°C for 2 days. The percent survival was then calculated.

Tetrads (spores in ascus) vs. free spores



Heterozygous strain (ZKY1)

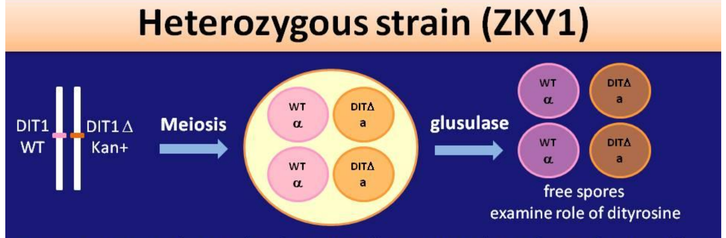


Figure 6 – Spores in the tetrad maybe protected against UV radiation due to a layering effect within the ascus. UV can damage spores sitting on the surface but is not able to penetrate and reach the spores on the bottom layer. Diploids produced by opposite mating types are able to grown making it difficult to accurately measure the protective role of dityrosine. To remedy this problem, the ascus was digested with glusulase in order to release the free spores enabling us to assess the role of dityrosine without the protection of the ascus. In this experiment, we used the heterozygous DIT1 mutant strain (ZKY1) where one copy of the DIT1 gene is knocked out by the insertion of the kanamycin antibiotic resistance gene.

ZKY1 - % Survival of Tetrads following UV exposure at 254 and 302 nm

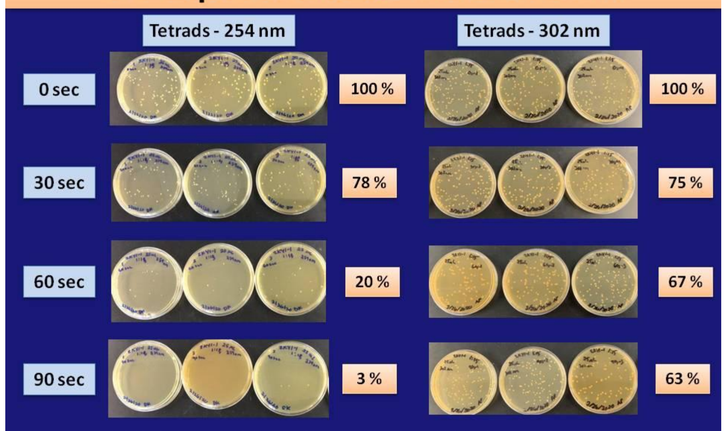


Figure 7 – Tetrads (spores in the ascus) were plated on YAPD plates and were exposed to UV radiation at 254nm and 302nm, respectively and the % survival was calculated. The % survival after 90 sec is only 3% at 254 nm compared to 63% survival at 302 nm.

ZKY1 - % Survival of Tetrads following UV exposure at 254 and 302 nm

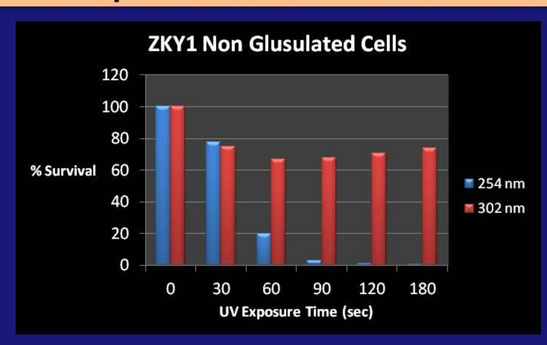


Figure 8 – UV exposure at 302 nm results in higher survival rates for the tetrads. Dityrosine has an absorption spectrum at 300-320 nm which could explain these results. Even following a 3 min UV exposure at 302 nm, 74% of the tetrads survived.

ZKY1 - % Survival of Free spores following UV exposure at 254 and 302 nm

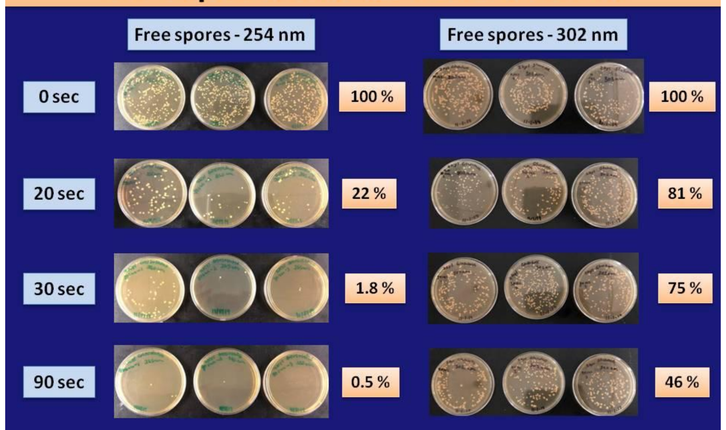


Figure 9 – The glusulated tetrads (free spores) were exposed to UV radiation at 254 and 302 nm and the % survival was calculated. The % survival after 90 sec is only 0.5% at 254nm compared to 46% survival at 302 nm. Spores had a better survival rate at 302 nm however free spores are more susceptible to UV radiation.

ZKY1 - % Survival of Free spores following UV exposure at 254 and 302 nm

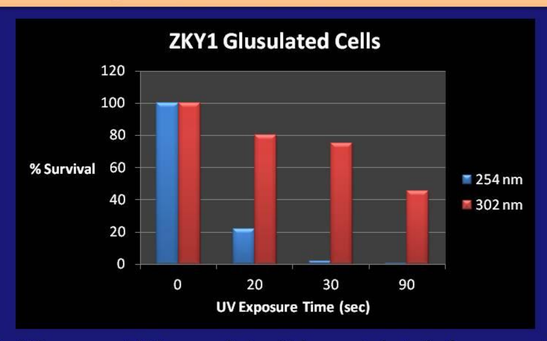


Figure 10 – UV exposure at 302 nm results in a higher survival rate for free spores. In addition, free spores are more susceptible to UV radiation. This demonstrates how the layering effect of the ascus plays a significant role in protecting spores from UV radiation.

Conclusion

- Tetrads exposed to UV radiation at 254 nm were more susceptible than tetrads exposed at 302 nm. Only 3% survival was observed after a 90 sec UV exposure at 254 nm compared to 63% survival at 302 nm.
- Treatment with glusulase to release the spores from the ascus of the ZKY1 DIT1 heterozygous strain resulted in free spores that were highly sensitive to UV light. Only 0.5% survival was observed after a 90 sec UV exposure at 254 nm compared to 46% survival at 302nm. This suggests that the ascus provides some protection by allowing spores to hide from UV exposure.
- Survival for both tetrads and free spores was higher at 302 nm. Dityrosine has an absorption spectrum at 300-320 nm which could explain the difference in the survival rates. This range corresponds to the part of the total spectrum at sea level that is responsible for the mutagenic action of sunlight.

References

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- Peter Briza, Michael Breitenbach, Adi Ellinger, and Jacqueline Segall (1990). Isolation of two developmentally regulated genes involved in spore wall maturation in *Saccharomyces cerevisiae*, Genes and Development, 4:1775-1789