Probing Molecular Mechanisms of Radioresistance: Toward Tunable Pigmentation for Passive Fungal Sensor Arrays of Radiation Exposure

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Goals & Outcomes

- Investigate the role of melanin in radioresistance
- Identify biological signatures of radioresistance
- Exploit biochemical mechanism of radioresistance to develop a low-cost, passive radiological biosensor

Introduction

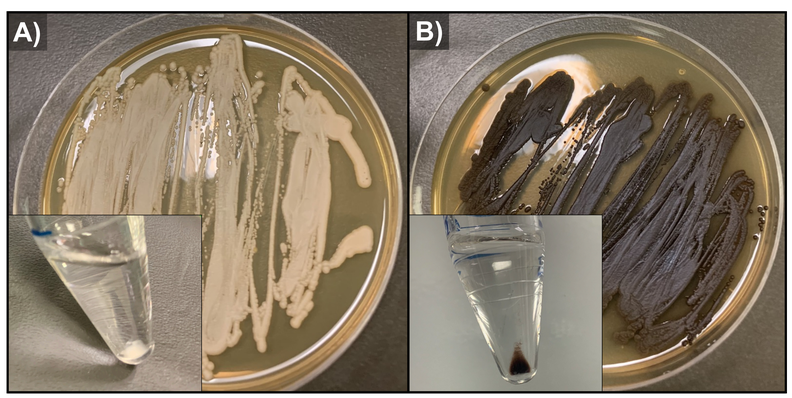
History of Radioresistance

Microorganisms were discovered growing at the Chernobyl reactor in 1991. One of the colonizing species, *Exophiala dermatitidis*, a melanized fungus, exhibits increased growth rates in radiative conditions. This radioresistant phenotype is referred to as *radiotropism*. While this is a well documented phenomenon, the molecular machinery utilized to proliferate under such harsh conditions has not been identified.

Exophiala dermatitidis as a Model Organism

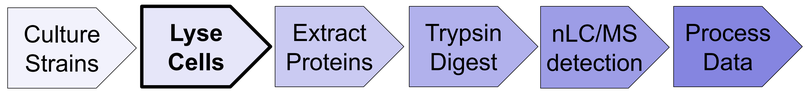
Since *Exophiala dermatitidis* has been highly studied and the genome fully sequenced, it is a suitable model organism for radiotropic species. *E. dermatitidis* is a melanin-producing fungus that stores 1,8-dihydroxynapthalene in it's cell walls. An albino mutant strain was obtained by deletion of the WdPKS1 gene integral to a melanin-producing pathway. Initial comparative experiments suggest that melanin is essential to radiotropism since the albino mutant is not radioresistant. However, transcriptomic studies proved inconclusive in identifying gene products linked to radioresistance between the two strains.

Figure 1: A) Albino ΔPKS mutant and B) wild type *Exophiala dermatitidis* cultures. The plates were incubated at 37 °C for 5 days. The inset images show the pigmentation for the two strains more clearly when cells are suspended in protein extraction buffer. The ΔPKS mutant has no appreciable levels of pigmentation whereas the wild type is dark brown/black.



Methods

A full analysis method was developed using *E. coli* to be adapted to the *E. dermatitidis* strains. Both the wild type black fungus and albino ΔPKS mutant were obtained in June of 2021. Since fungi like *E. dermatitidis* have particularly robust cell walls, traditional lyses methods can be challenging to extract reproducible amounts of proteins. Several combinations of lyses techniques were investigated to obtain reasonable protein recovery.

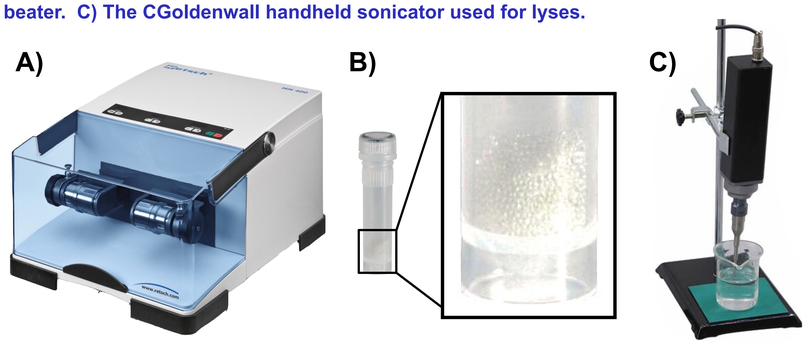


Freeze/thaw cycles were done by alternating between dry ice and boiling water 5 times for at least 2 minutes in each. Each tube is visually confirmed to be totally frozen/thawed before switching.

Bead beating was done with a Retsch MM440 at the maximum settings using 2 mL tubes with 0.5 mm glass beads.

Sonication was done 5 times for 5 seconds each. Between each round, the vials were kept on ice for 2 minutes to prevent vials from heating and subsequent protein degradation.

Figure 2: A) Image of the Retsch bead beater. B) Close of image of the beads in the purchased tubes. Cell suspensions are added to the tubes and agitated in the bead beater. C) The CGoldenwall handheld sonicator used for lyses.

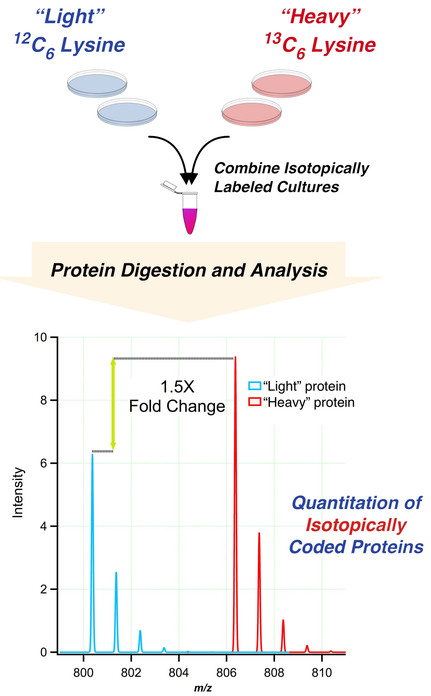


Results

The lysis method used for *E. coli* method development was sonication generating ample protein content and suitable proteome coverage. However, even a combination of freeze/thaw and sonication samples did not return a detectable amount of protein for *E. dermatitidis.* This prompted the use of the bead beater for cell lysis, and the data are still in progress.

Next Steps

Recovered proteins from the two strains will be analyzed using a bottom-up proteomics workflow. Comparisons between the melanized, radioresistant wild type and albino mutant will be made. Other mutant strains will be created by genetically modifying the wild type. Proteins affected by the transformations made in response to experimental conditions using SILAC may yield understanding of biological signatures of radiotropism.



Conclusions

A comprehensive body of work has yet to be assembled for E. dermatitidis and understanding radioresistant behavior. Comparative proteomics of the melanized wild type and albino mutant strains grown in several environmental conditions may elucidate possible biomarkers of interest. Stock solutions of the two strains have been successfully cultured as WSU and will be compare phenotypic differences derived from the proteome. Unique signatures to the wild type culture may be exploited for biosensor development that is sensitive and cost effective.

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