Exploring Protein Expression in *Exophiala dermatitidis* to Identify Biological Mechanisms of Radioprotection

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Goals & Objectives

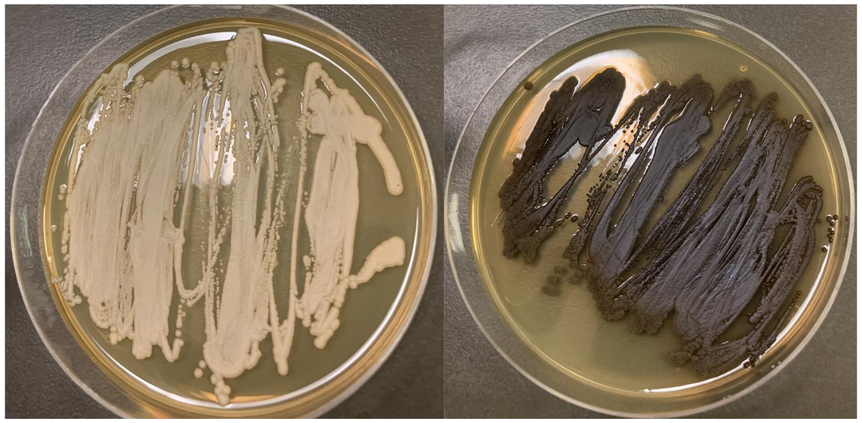
- Investigate the role of melanin in radioresistance
- Identify proteomic signatures of radioresistance
- Utilize signatures to develop a low-cost, radiological biosensor

Introduction

***Exophiala dermatitidis:* a Model Organism of Radiotropism**

Microorganisms were discovered growing towards the Chernobyl reactors. 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 a well documented phenomenon, the molecular machinery utilized to proliferate under such harsh conditions has not been identified.

Figure 1: A) Albino ΔPKS mutant and B) wild type *Exophiala dermatitidis* cultures. The plates were incubated at 37 ° C for 5 days. The wild type shows the dark pigmentation associated with melanized fungi while the ΔPKS mutant shows negligible pigmentation.



Proteomics to Investigate Phenotypic Differences

Since *E. dermatitidis*, as a well known human pathogen, has been highly studied and the genome fully sequenced, it is a suitable model organism for radiotropic species. However, as genes are only the instructions of a cell, genomic studies are insufficient for understanding how cells are responding to their environment. Proteins, a type of primary actors within a cell, are of high interest in explaining phenotypic phenomenon. By generating a theoretical proteome based on the genetic database, experimentally detected proteins can be matched and analyzed. Differences in the strains' suite of proteins can be used to explain differences in radioresistance.

Methods

A full analysis method was developed using *E. coli* and adapted to the *E. dermatitidis* strains. Both the wild type black fungus and albino ΔPKS mutant have been cultured at WSU. Cells are lysed using a bead beater and proteins extracted by dissolving in ammonium acetate and urea. The proteins are then digested using a standard bottom-up proteomics approach. Proteins are enzymatically cut, chemically modified, and measured using liquid chromatography-mass spectrometry. The theoretical proteome is used to match the detected fragments and reassemble intact proteins.

A modified procedure for utilizing cells from solid media was developed for future experimentation on cells outside of liquid media. New materials specific to protein workflows were used to decrease protein loss in processing stages. Optimized LC/MS methods for greater separations of proteins were tested to increase the number of proteins detected.

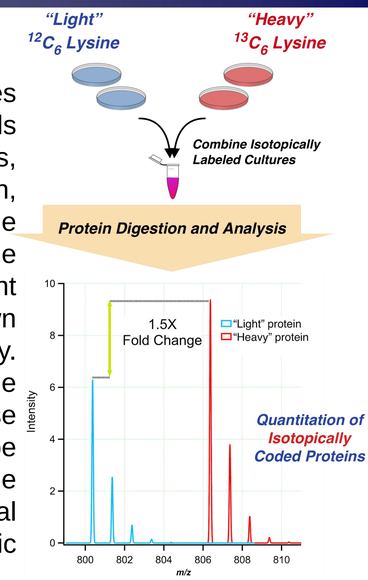
Results

	Total Proteins	Number of Related Proteins: Oxidative Stress Response	Stress Response	Oxygen and Radical Detoxification
Wild Type (Melanized)	1710	40	119	13
Δpks Variant (Non-Melanized)	2685	46	150	16

The new method for obtaining proteins from cells grown on solid media resulted in more than 2800 proteins being identified, more than have ever been reported for *E. dermatitidis.* This method involves extracting cells from colonies grown on solid media plates and following our previous protein extraction protocol. This is a four-fold increase over what has been reported. Unlike our previous work, the albino mutant samples generated more detected proteins than the wild type samples. This is likely due to melanin in the wild type samples obstructing the cell lysis and protein extraction. One protein of note, DNA damage-binding protein, was found in much higher abundance in the albino mutant samples. This protein is responsible for repairing UV-damaged DNA, so a higher abundance in the albino mutant may indicate the DNA in these cells are experiencing a higher amount of UV damage because a lack of protective melanin. This example highlights the utility of proteomic studies in determining the ongoing cellular functions, and will be useful when comparing the effects of different types of stresses on the cells.

Next Steps

Studies on proteomic differences stemming from alternate methods for introducing oxidative stress, like peroxides and UV radiation, will be conducted to separate the effects of typical oxidative damage from radiative damage. Current attempts to replicate known experiments are underway. Proteins affected by the transformations made in response to experimental conditions can be detected using SILAC to enable the understanding of biological signatures of radiotropic organisms.



Conclusions

A comprehensive body of work has yet to be assembled for *E. dermatitidis* and radiotropic or radioresistant behavior. Comparative proteomics of the melanized wild type and albino mutant strains grown in several environmental conditions may elucidate possible biomarkers of interest. Further studies are being conducted to compare proteomic changes from various stresses on wild type and albino mutants of *E. dermatitidis* to determine how melanin production is related to radioresistance and radiotropism. Unique signatures to the wild type culture may be exploited for biosensor development that is sensitive and cost effective.

Bibliography

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