Probing Molecular Mechanisms of Radioresistance: Toward a Passive Fungal Sensor Array

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Goals & Objectives

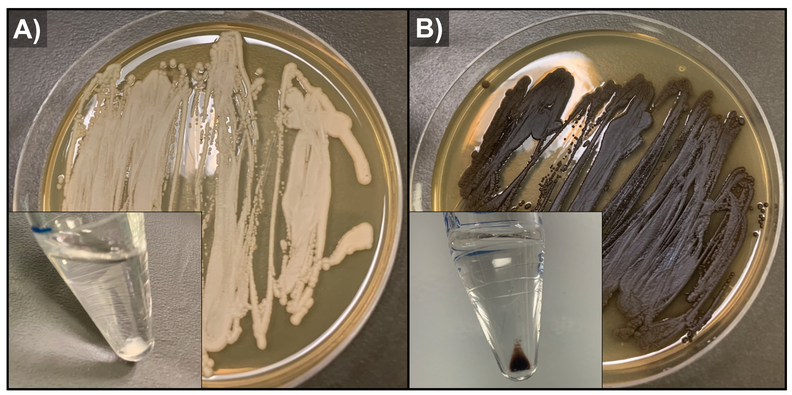
- Investigate the role of melanin in radioresistance
- Identify biological signatures of radioresistance
- Utilize signatures to develop a low-cost, radiological biosensor

Introduction

Exophiala dermatitidis: a Model Organism of Radiotropism

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 inset images show the two strains 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.

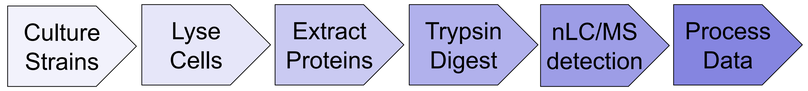


Proteomics to Investigate Phenotypic Differences

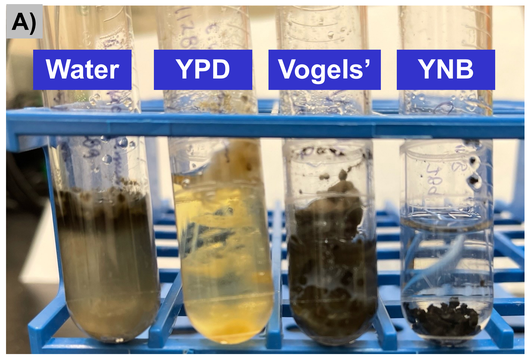
Since *E. dermatitidis* has been highly studied and the genome fully sequenced, it is a suitable model organism for radiotropic species. Proteins, the actors within a cell, are of high interest in explaining phenotypic phenomenon. By generating a theoretical proteome based on the genetic database, experimentally 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 for *E. dermatitidis* strains. Both the wild type black fungus and albino ΔPKS mutant have been cultured and are under investigation at WSU.



In conjunction with PNNL, a suite of genetically transformed strains using *Cladosporium sphaerospermum,* another model melanized fungus with less toxicity but less studies, have been attempted as a first pass. By using conserved regions in the genome, investigation in utility of the transformations can begin using ribonucleoprotein (RNP) transformation. Three transformations were attempted by electroporating *C. sphaerospermum* with the RNP.



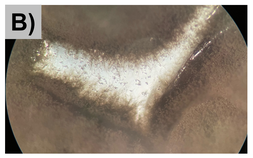


Figure 2: A) Growing in different medias changes the pigmentation of *C. sphaerospermum*. YNB is favorable for colonial growth making it easy to select mutant colonies. B) Pigmented tissue of *C. sphaerospermum*.

Results

Lys2: lysine auxotroph for SILAC. Survival was expected in aminoadipic acid (AAA), but did not prove to be a selective target.

Ura3: deletion of Ura3 enzyme. Survival in 5-fluroorotic acid (5-FOA) is expected since the toxic metabolite can no longer be produced.

PKS1: gene deleted for *E. dermatitidis* albino mutant. Less pigment is expected than without transformation

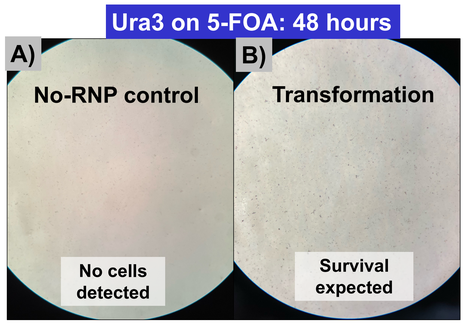


Figure 3: Testing RNP uptake of Ura3 by plating A) the control colonies on toxic 5-FOA and B) the colonies exposed to RNP. After 48 hours, it appears no colonies are growing without the Ura3 exposure while some cells have appeared after transformation. This indicates some successful uptake of the Ura3 gene. Isotopically labeled 5-FOA can be used to monitor what proteins respond to exposure to stressors.

Next Steps

Proteomics of *E. dermatitidis*: Mutant vs. Melanized Strains

In order to obtain quantitative, reproducible results, growth of the strains must be standardized for cell counts. Once the data is collected, the theoretical proteome must be annotated for tangible phenotypical inferences.

RNP Transformation Evaluation

After just 48 hours, viability of the fungus on 5-FOA after Ura3 transformation illustrates reasonable success. The Lys2 mutant scheme must be revisited to evaluate if AAA is a suitable choice to test viability. PKS1 mutants will be analyzed under a microscope to evaluate differences in pigmentation produced.

Specificity studies using *C. sphaerospermum* and H_2O_2

While the goal of these experiments is to expose *E. dermatitidis* to ionizing radiation for biosensor development, specificity into the response to ionizing radiation and analogous genes must be investigated. We plan to compare exposure response from hydrogen peroxide (H_2O_2) to ionizing radiation as well as transformations in the two species.

Conclusions/relevance to TA3

By understanding the specificity of toxic oxidative environments on two similar fungi, select gene targets can be identified for an array of strains with varying radioresistance. Since pigmentation seems to be linked to the resistance, a tunable pigmentation biosensor could be a low-cost, passive sensor to radiation.

Biological response to radiation occurs within one hour. By simply testing for viability after exposure, the dosage of radiation can be quantified by understanding the resistance of each strain

Bibliography

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