Probing Molecular Mechanisms of Radioresistance in Melanized Fungus

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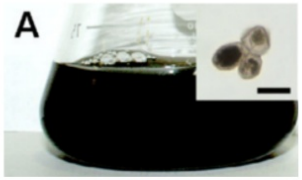
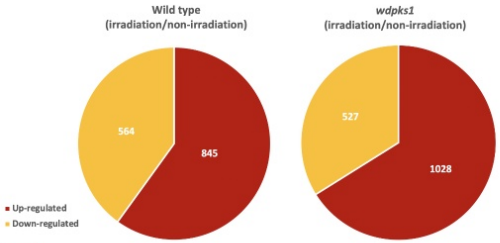
Pacific Northwest NATIONAL LABORATORY

Discovery of Radiotropism



Contradicting Results

Bottom-up Proteomics



Goals & Outcomes

(1) Washington State University
(2) Pacific Northwest National Laboratory

References:

ETI

Discovery of Radioresistance

- **Microorganisms discovered at Chernobyl in 1991**
- ***Wangiella dermatitidis* (*Exophiala dermatitidis*)**
  - Melanin deposited in the cell wall
  - Full genome sequenced
  - Proteome remains unexplored
- **Early investigations demonstrated increased growth rates when exposed to radiation**
- **Radiotropism**
  - Characteristic of organisms to proliferate in radiative environments

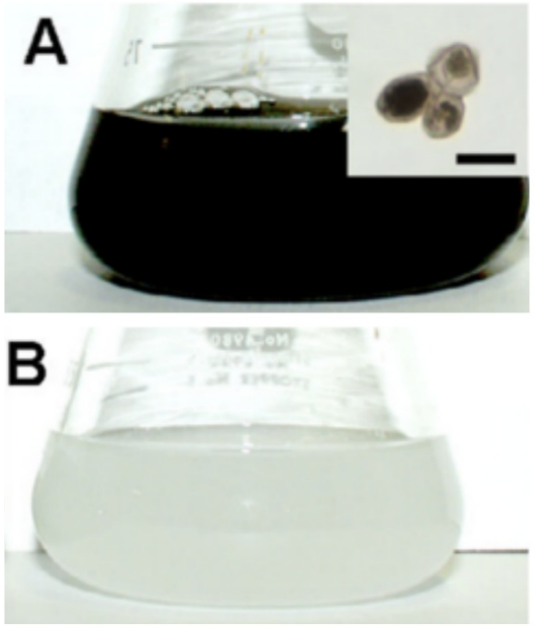
Contradicting Results

Dadachova *et al. 2007.*

- Irradiated *W. dermatitidis* colonies with and without melanin (albino mutant)
- Observed that melanized colonies were resistant to much higher levels of radiation
- **Conclusion: Melanin protects fungi from ionizing radiation**
  - free radical quenching, possible energy capture

Robertson *et al. 2012.*

- Transcriptomic study of gene response to ionizing radiation
- Saw only 5% of differentially regulated genes were melanin specific
- **Conclusion: Radiotropism stems from components other than melanin**



A: Melanized *W. dermatitidis*
B: Albino mutant with no appreciable levels of melanin

Bottom-Up Proteomics

- **Proteins are relatively large molecular species**
  - Analysis of intact proteins presents challenges
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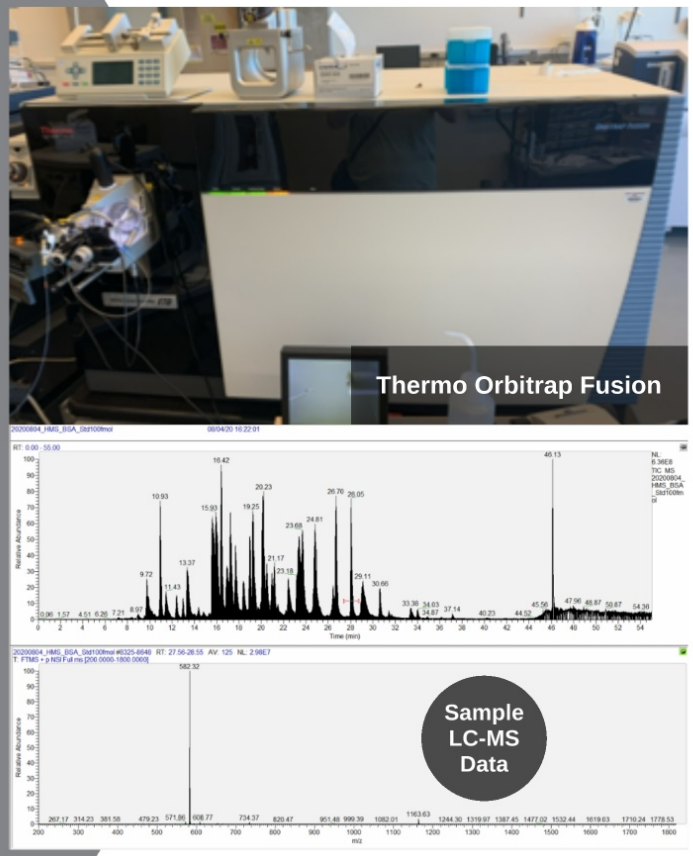
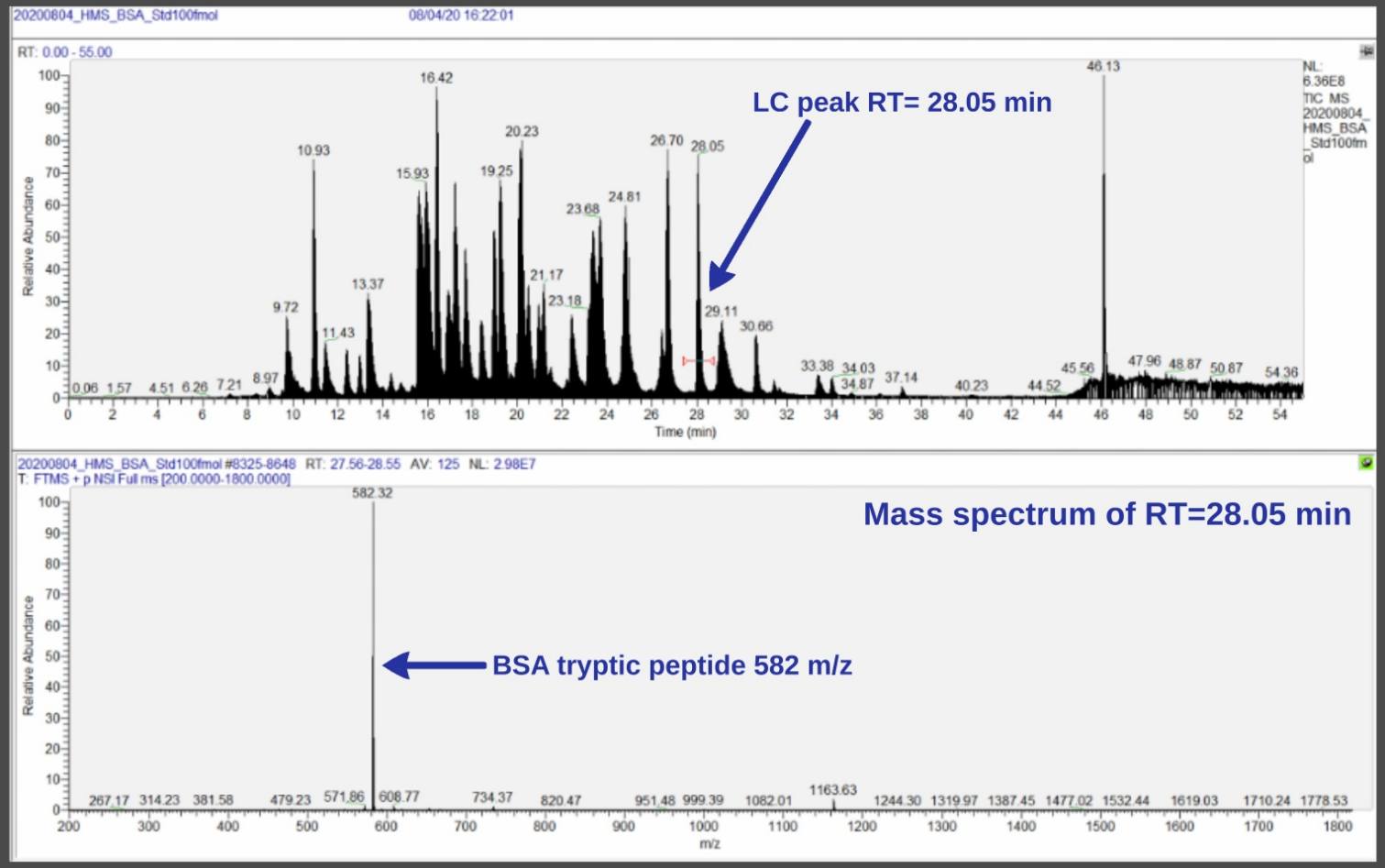
- **Bottom-up utilizes protease to break down proteins**
  - Trypsin: selective at Lys and Arg residues
- **Digested peptides are analyzed with liquid chromatography then mass spectrometry**
- **Reassembling the puzzle pieces of the proteome**



100 fmol Tryptic Digest of Bovine Serum Albumin



Goals and Outcomes

Year 1: 2020

- **Train on sample preparation and instrument protocols**
- **Obtain wild type, melanized *W. dermatitidis* and albino mutant**
- **Method developments**
  - Sample analysis
  - Informatics
- **Compare proteome of wild type and albino mutant**

Future Directions

- **Sample irradiation**
  - Different sources
  - Chronic vs. dosed exposure
- **Identify biological signatures**
  - Comparing proteome results
  - Quantitative proteomics
- **Develop a biosensor**
  - Low cost
  - Exploit identitfied biological signatures

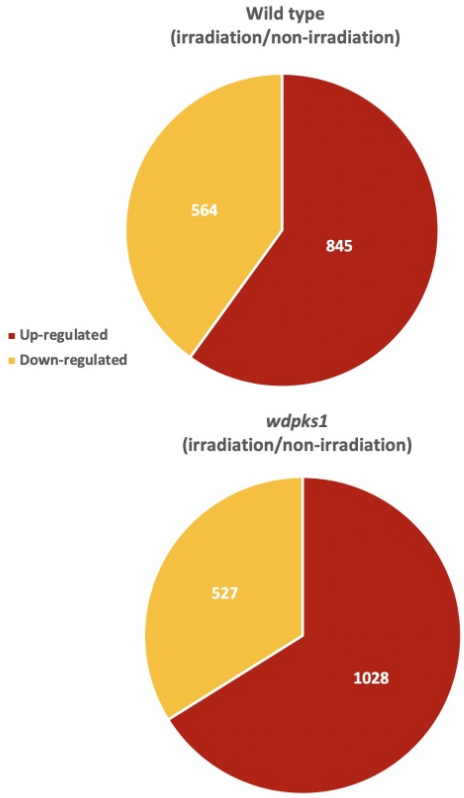


Figure adapted from Robertson *et al:* Comparing the number of differentially regulated genes after exposure to radiation for melanized wild type *W. dermatitidis* and its albino mutant, *wdpk1*.

References

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Daly, MJ. *Death by protein damage in irradiated cells.* **2011**. doi:10.1016/j.dnarep.2011.10.024

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