Oxidative Extremotolerance of *Acinetobacter radioresistens* 50v1, a Gram-Negative Bacterium Isolated from the Mars Odyssey Spacecraft

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Kellogg Honors College Capstone Project

Objectives

- How do microbes survive in s/c assembly facilities?
- How do oligotrophic conditions impact extremotolerance?
- Does oxidative stress impact the microbes metabolome?

Background

- Spacecraft-associated Microorganisms (SAM):
  - Surfaces of preflight spacecraft, bench tops, floors…
- Ethanol and isopropanol (IPA) are cleaning solvents.
- *Acinetobacter* are common SAM:
  - *A. radioresistens* 50v1 (surface of Mars Odyssey)
- Extremotolerant towards H_2O_2 (nutrient rich media, LB)

Methods

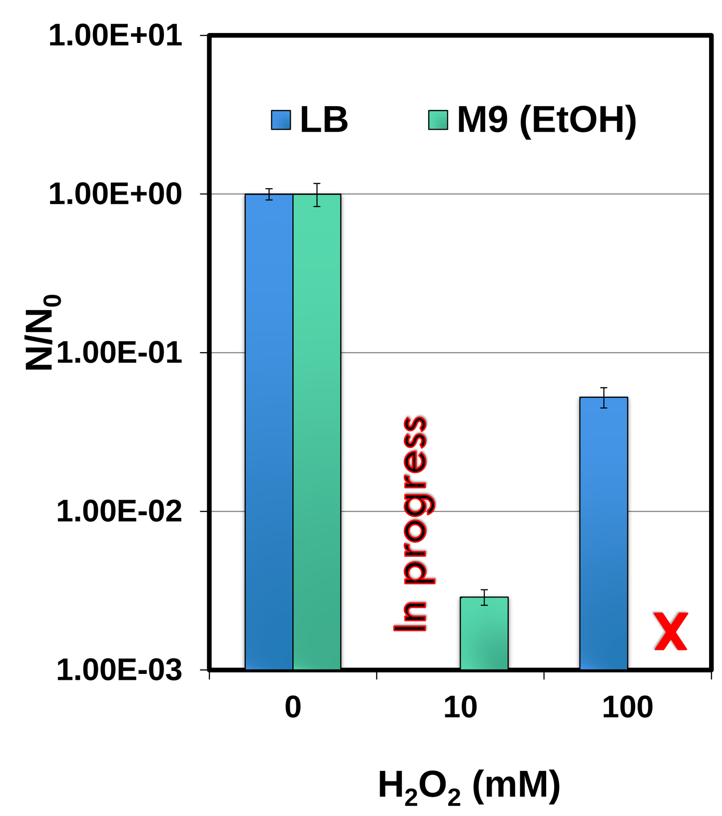
- Nutrient Poor vs. Rich Media
- Nutrient Poor: Minimal conditions, 0.2xM9, 26 mM Fe^{2+}
- Carbon sources: 0.1% mM ethanol
- Viability assays: Plate Counts
- Extremotolerance: 1-100 mM H_2O_2 in M9/EtOH/Fe & LB
- Metabolomics: GC-MS of 1mM H_2O_2 exposures…
- GC-MS using Agilent MSD
- Extraction using 1:1 $AcCN/H_2O$…
- Derivatization using CH_3ONH_2 and/or MSTFA…
- Metabolite Analysis: Compared using NIST library…

Summary of Results

- 100 mM H_2O_2 LB: ~1.0-log reduction from ~10^9 cfu/mL
- 10 mM H_2O_2 in M9: ~2.5-log reduction from ~10^8 cfu/mL
- 100 mM H_2O_2 in M9: Total loss of *A. rad.* 50v1
- Aspartic Acid, isoleucine, cysteine, tryptophan, valine, lysine, and methionine all increased in abundance
- Tyrosine, glutamine, and glutamic acid all decreased in abundance
- Sucrose and trehalose increased in abundance
- Fructose and mannose decreased in abundance
- Certain fatty acids decrease in abundance (C_{10}, C_{16}, & C_{18})

Oxidative Extremotolerance on Nutrient Rich vs. Poor Media

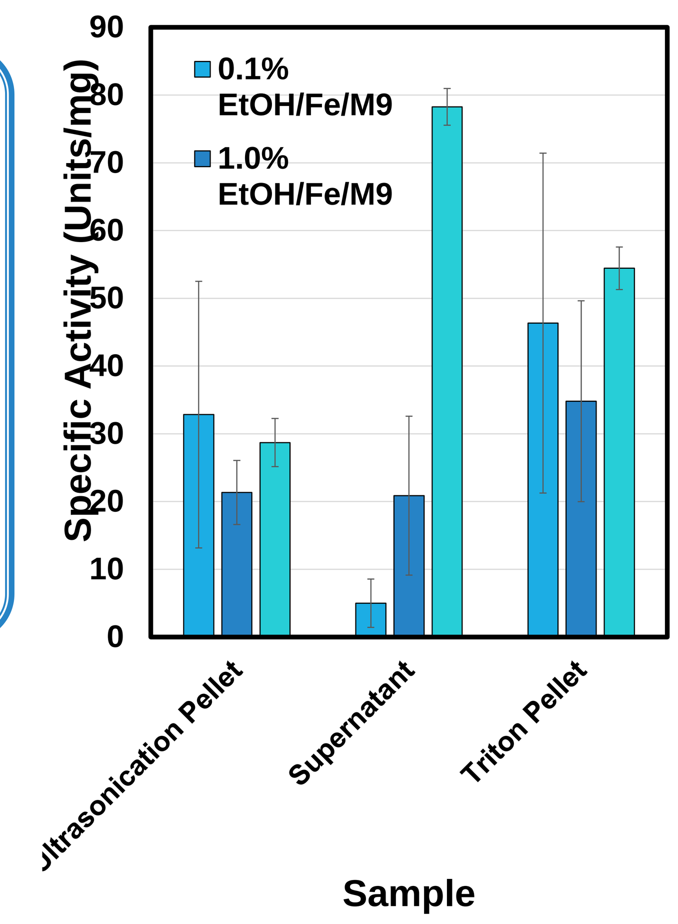
H_2O_2 Exposure (0-100 mM)
LB vs. 0.2xM9, 16 mM EtOH, 26 µM Fe^{2+}
A. radioresistens 50v1



Results
- Survivability of H_2O_2 Exposure…
- LB:
  - ~1.0 log loss in 100 mM
- M9:
  - ~2.5 log loss in 10 mM
  - Total loss in 100 mM

Kim Sripong and Alexa Campos

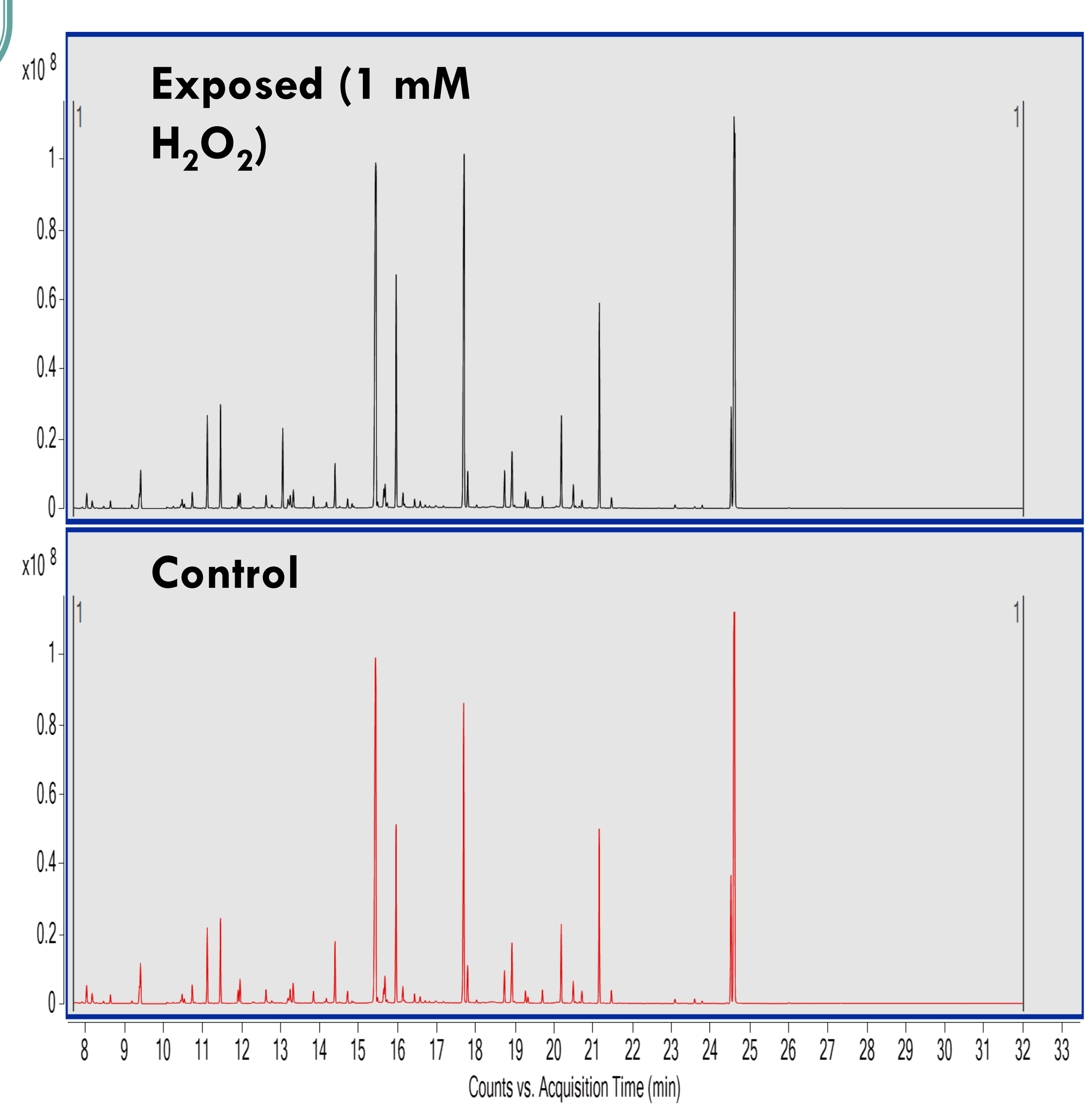
Catalase Specific Activity
Impact of Culture Conditions
A. rad. 50v1 (M9/EtOH/Fe v. LB)

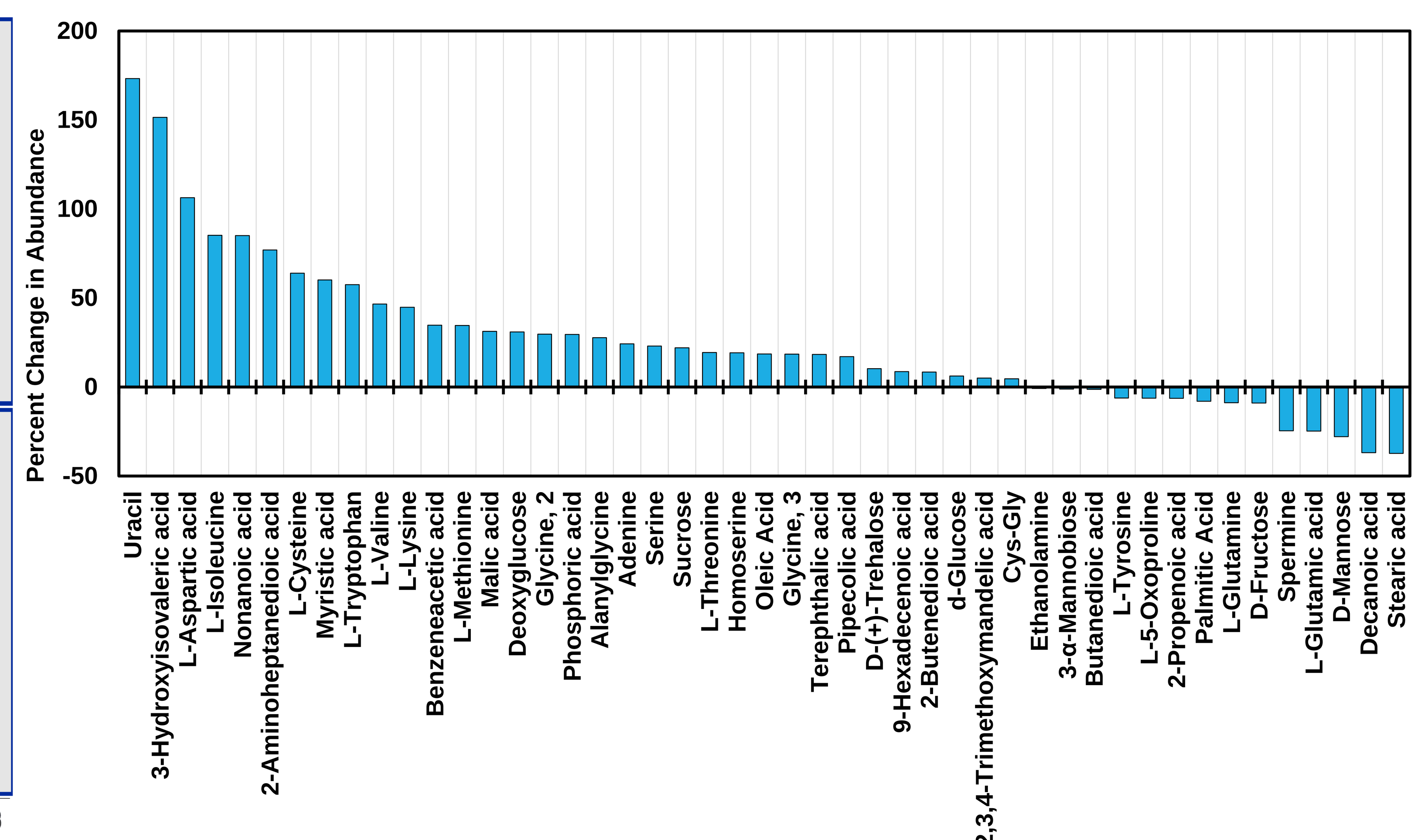


Results
- Catalase degrades H_2O_2 (into H_2O + O_2)…
- Catalase Specific Activities (20 mM H_2O_2):
  - LB > M9/EtOH
  - LB: Supernatant > Pellet
  - M9: Pellet > Supernatant
  - Increase [C] : Increase catalase SA

Sooji Lee

Metabolomics of 1 mM H_2O_2 Exposure in LB Media





Conclusions

- **A. *radioresistens* 50v1 is extremotolerant towards H_2O_2 under oligotrophic conditions.**
- **This suggests that SAM display oxidative extremotolerance under cleanroom conditions.**
- **Catalase is important step in oxidative extremotolerance…**
- **Under nutrient rich conditions, catalase is mostly solube in the cell…**
- **Under nutrient poor conditions, catalase is mostly membrane bound…**
- **Upon exposure, biosynthesis of disaccharides used in oxidative stress increases…**
- **Upon exposure, biosynthesis of oxidizable amino acids increases…**
- **Upon exposure, biosynthesis of "compatible solute" amino acids changes (Asp vs. Glu)…**
- **Next steps include:**
  - **Survivability in 10 mM H_2O_2 in LB (nutrient rich)…**
  - **Metabolomics of 1 mM H_2O_2 exposures in M9/EtOH (nutrient poor)…**

References

[1] Space Studies Board (2006) Preventing the Forward Contamination of Mars National Academies Press, Washington DC. [2] McCoy et al. (2012) Astrobiology 12:854-862. [3] Derecho et al. (2014) Astrobiology 14:837-847.

Acknowledgements

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