Impact of Carbon Sources on the Metabolomics and Extremotolerance of Spacecraft-Associated *Acinetobacter*

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Objectives

- How do microbes survive in s/c assembly facilities?
- Are spacecraft cleaning solvents carbon sources?
- Are spacecraft cleaning solvents energy sources?
- How do oligotrophic conditions impact extremotolerance?

Background

- Spacecraft-associated Microorganisms (SAM):
  - Surfaces of preflight spacecraft, benchtops, floors, etc.
- Ethanol and isopropanol (IPA) are cleaning solvents.
- Acinetobacter are common SAM:
  - *A. radioresistens* 50v1 (surface of Mars Odyssey)
  - *Acinetobacter* spp. (floor of Mars Phoenix facility)
- Extremotolerant towards H_2O_2 (in nutrient rich media, LB):
  - *A. rad.* 50v1 (no loss in 100 mM H_2O_2)
  - *A. gyllenbergii* 2P01AA (no loss in 100 mM H_2O_2)

Methods

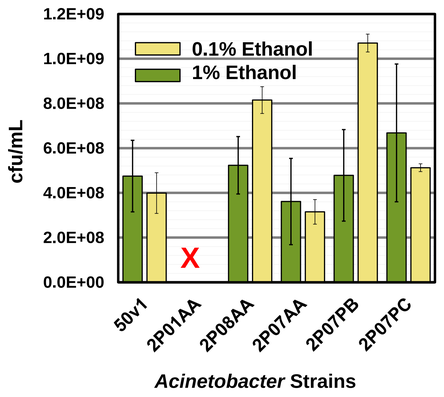
- Minimal culture conditions (M9) with 26 µM Fe^{2+}
- Carbon sources: 0- 200 mM ethanol and/or IPA
- Viability assays: Plate Counts & Optical Density
- Extremotolerance: 0-2 M H_2O_2 in M9/EtOH/Fe & LB
- Cellular Extractions: Bugbuster & Ultrasonication
- Alcohol Dehydrogenase: UV-Vis; NAD^+, DCIP (pH 7.5)
- Catalase: UV; 20 mM H_2O_2 (pH 7.5)
- GC-MS: EtOH v. EtOD, M9/Fe; Agilent MSD
- NMR: M9/EtOH v. LB; 400 MHz Varian NMR

Results

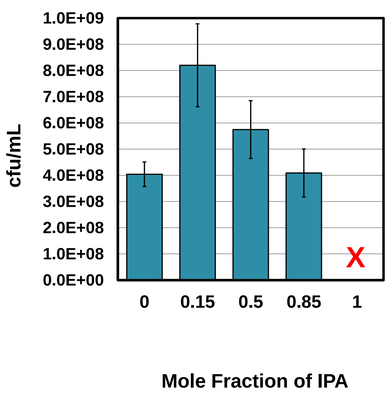
- Most s/c-assoc. *Acinetobacter* grow on EtOH (M9/Fe)…
- Growth rates higher in 16 mM EtOH (v. 160 mM)…
- IPA is not a sole carbon source (under these conditions)…
- IPA does enhance growth in EtOH mixtures…
- Oligotrophic cultures are still extremotolerant…
- EtOH/M9 cultures: ~2-log reduction, 100 mM H_2O_2
- *Extremotolerance*: LB cultures > EtOH/M9 cultures
- ADH & catalase activities are mostly in pellet fractions…
- ADH is PQQ & NAD^+-dependent (DCIP redox couple)…
- K_m (app) ~ 6 mM EtOH
- *ADH Specific Activities*: EtOH/M9 cultures > LB cultures
- *Catalase Specific Activities*: EtOH/M9 > LB
- GC-MS supports biosynthesis using ^{2}H-EtOH (EtOD)!!
- NMR supports biosynthesis using ^{1}H-EtOH!!

• METABOLISM OF SPACECRAFT CLEANING SOLVENTS

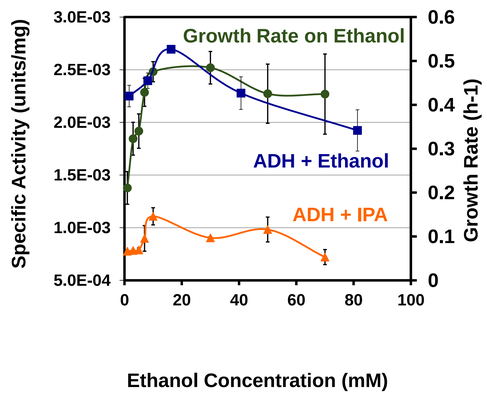
Viability in 0.1% and 1% EtOH
(16 & 160 mM EtOH, 26 µM Fe^{2+}, M9)



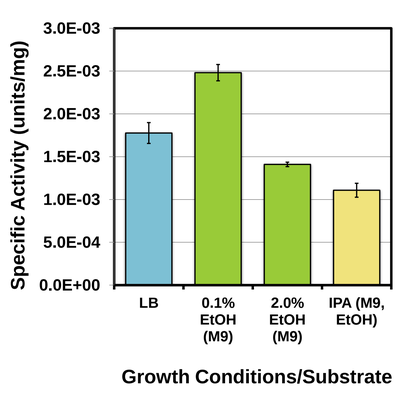
Viability on IPA Mixtures
200 mM Total Mixtures
p<0.05 (0.15 & 0.5 v. no IPA)



Alcohol Dehydrogenase
Michaelis-Menten Plots (EtOH v. IPA)
PQQ & NAD⁺-dependent ADH (extract)

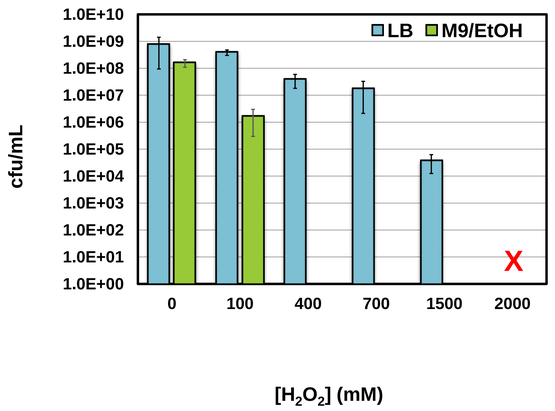


ADH Specific Activities
Impact of Culture Conditions
LB; 0.1% & 2% EtOH, 0.1% IPA (M9)

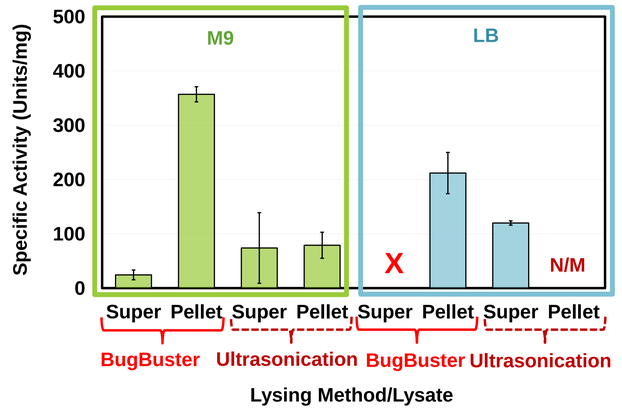


• OXIDATIVE EXTREMOTOLERANCE ON H_2O_2

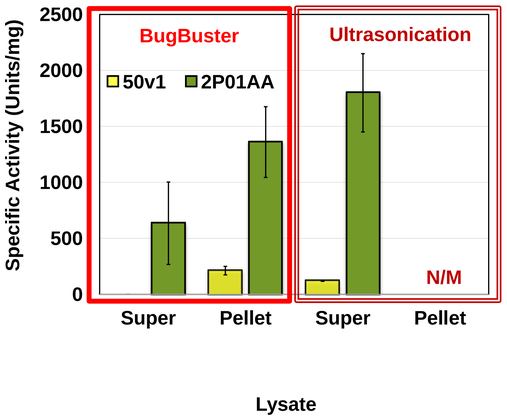
Survival Under H_2O_2 Exposure
Impact of Culture Conditions
A. radioresistens 50v1 (M9/EtOH/Fe v. LB)



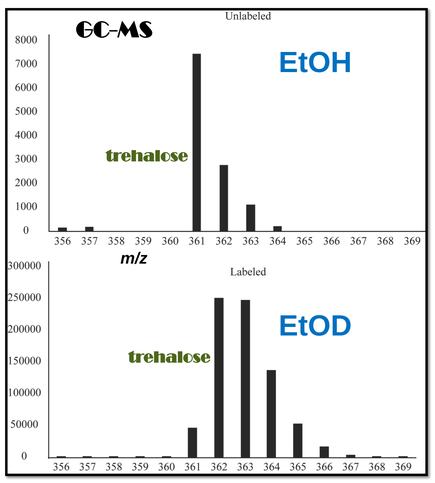
Catalase Specific Activity
Impact of Culture Conditions
A. rad. 50v1 (M9/EtOH/Fe v. LB)



Catalase Specific Activity
Impact of Lysing Methods
50v1 & 2P01AA (LB)



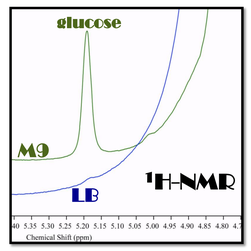
• ETHANOL IS A CARBON SOURCE



A. radioresistens 50v1

GC-MS
- ^{1}H-EtOH v. ^{2}H-EtOH (in M9/Fe)…
- Cells extracted with 50/50 AcCN/H_2O…
- AcCN extract dried & derivatized w/ MSTFA…
- GC-MS analysis…
- MS spectra of ^{1}H & ^{2}H-trehalose (similar retention times)…
- *m/z* differences support deuterium labeling…

NMR
- ^{1}H-EtOH in M9/Fe & LB…
- Cells washed in PBS…
- Resuspended in H_2O/D_2O…
- ^{1}H-NMR obtained…
- Chemical shift of glucose…
- Example of biosynthesis…



Conclusions

- **EtOH is a carbon & energy source for SAM.**
- **ADH & catalase are membrane-bound.**
- **Spacecraft cleaning solvents may impact microbial ecology of assembly facilities…**
- **Planetary Protection practices may need to consider impact of the cleaning solvents…**

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.