Metabolomics and Oxidative Extremotolerance of Spacecraft-Associated *Acinetobacter* Grown on Ethanol. R. Baki[1], S. Lee[1], A. Campos[1], N. Perkins[1], G. A. Barding[1], and R. Mogul[1], [1] Chemistry and Biochemistry Department, Cal Poly Pomona, 3801 West Temple Avenue, Pomona, CA 91768 (rmogul@cpp.edu).

Introduction: Protecting Mars from biological contamination is critical to ensuring the integrity of future life-detection missions. Despite the stringent cleaning protocols for Mars-bound spacecraft, there remains a persistent bioburden within the cleanroom facilities where spacecraft are assembled. Typically, these spacecraft-associated isolates are tolerant towards oxidative, radiation, and desiccating conditions, and hence carry the potential to survive or persist on/in spacecraft during Mars exploration, and perhaps in the martian regolith [1]. In this presentation, we will provide molecular and biological evidence that *Acinetobacter radioresistens* 50v1, which was isolated from the preflight Mars Odyssey orbiter, metabolizes/degrades ethanol and 2-propanol, which are used as spacecraft cleaning solvents.

Methods: Cultures of *A. radioresistens* 50v1 were prepared in nutrient rich lysogeny broth (LB) or nutrient poor minimal media (0.2xM9) containing 16-160 mM ethanol and 26 µM Fe^{2+}. Mid-log phase cultures were used for viability, enzymology, and metabolomics studies. Survivabilities (cfu/mL) were measured after exposures to 10, 20, and 100 mM H_2O_2 in both LB and M9/EtOH/Fe conditions. Alcohol dehydrogenase specific activites were measured by absorbance spectroscopy using cell extracts prepared through ultrasonication and centrifugation, whereupon both supernatants and pellets were assayed. Enzymatic assays surveyed multiple electron acceptor combinations (NAD^+, NADP, Fe^{3+}, DCIP, & XTT), inclusive of pseudo-Michaelis-Menten kinetic studies using ethanol and 2-propanol as substrates. Metabolomic studies were performed on cultures grown on ^{13}C-labeled ethanol (on postions C_1, C_2, and $C_{1,2}$) followed by GC-MS analysis of the cellular extracts.

Results: *Survivability*. Plate count assays support the use of ethanol as a sole carbon source by multiple strains of *Acinetobacter* isolated from the Mars Odyssey and Mars Phoenix facilities. Under oligotrophic conditions, *A. radioresistens* 50v demonstrates an appreciable oxidative extremotolerance, with exposures of 10 mM H_2O_2 yielding ~2-log reductions from ~10^8 cfu/mL (**Figure 1A**).

Enzymology. Specific activity measurements support the presence of a membrane-bound PQQ/NAD^+ dependent alcohol dehydrogenase (ADH) and the oxidation of both ethanol and 2-propanol. The ratio of membrane-bound to soluble ADH specific activities was culture-dependent, with cells grown in M9 showing higher membrane-bound activities. The comparison of electron acceptors and pseudo-Michaelis-Menten parameters for the alcohol substrates will be discussed (**Figure 1B**).

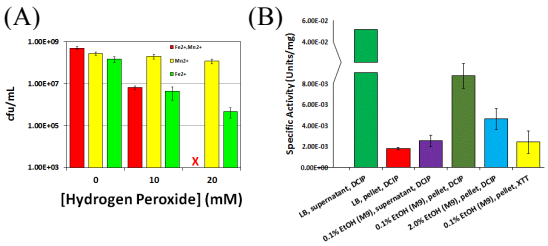

Figure 1A-B. (A) Oxidative extremotolerance of *A. radioresistens* 50v1 in H_2O_2; (B) Specific activities of Alcohol Dehydrogenase with varied growth media and terminal electron acceptors.

Metabolomics. Gas chromatography-mass spectrometry (GC-MS) studies confirm that ethanol is a sole carbon source under minimal conditions, with ^{13}C-labeled ethanol being incorporated into metabolites such as TCA/glyoxylate cycle intermediates, amino acids, monosaccharides, and disaccharides. Further, mixtures of 2-propanol and ethanol manifest reproducible changes in glutamate and aspartate abundances, which suggests metabolic adjustments to these alcohols. However, studies using per-dueterared 2-propanol and mixtures of 2-propanol and ^{13}C-ethanol show no substantial evidence of carbon acquisition from 2-propanol (under these conditions). The impact of label position and additional metabolic insights will be discussed.

Conclusion: Our student-centered work supports the metabolism and biodegradation of spacecraft cleaning solvents by the Mars-associated *Acinetobacter*. Hene, these results provide a potential biochemical rationale to the observed microbial ecology dynamics of spacecraft assembly facilities. Further, the observed oxidative extremotolerance (under oligotrophic conditions) support the potential for forward contaminantion of Mars by the *Acinetobacter*.

References: [1] McCoy, K. B., et al. (2012) *Astrobiology, 12,* 854-862. [2] Derecho, I., et al. (2014) *Astrobiology, 14,* 837-847.