Biodegradation of Kleenol-30 by *Acinetobacter radioresistens* 50v1, a bacterium isolated from the Mars Odyssey Spacecraft.

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Introduction: Proper cleaning and sterilizing procedures for spacecraft are of utmost importance when considering the probability of forward and backward contamination, including the integrity of life-detecting missions. To help ensure cleanliness, spacecraft are assembled in cleanroom facilities, where benchtop surfaces and floors are routinely wiped down with alcohols, detergents such as Kleenol-30, respectively. Despite these stringent cleaning protocols[1], spacecraft assembly facilities contain a persistent bioburden, with the *Acinetobacter* being among the most abundant genera. One such strain, *Acinetobacter radioresistens* 50v1 (50v1), was isolated from the surface of the preflight Mars Odyssey orbiter and found to be extremotolerant towards hydrogen peroxide, UV, and desiccation. In this presentation, we will provide molecular evidence that *A. radioresistens* 50v1 degrades and potentially metabolizes Kleenol-30, which is currently used as a cleaning reagent NASA facilties.

Methods: Mid-log phase cultures of *A. radioresistens* 50v1 were prepared in nutrient poor minimal media (0.2xM9) containing 16 mM ethanol and 26 µM Fe^{2+} in the absence and presence of 0.1% and 1.0% v/v Kleenol-30 (K30). Growth rates were measured by optical density (600 nm) and cultures were separated by centrifugation. Cell-free fractions (media broth, supernatant) were dried, extracted, derivatized by silylation, and analyzed by GC-MS. Metabolomic profiles were identified by comparison to the NIST library using Agilent MassHunter and AMDIS software.

Results: Analysis of the extracellular metabolites showed significant changes in the constituents of K30 and *A. radioresistens* 50v1. In the presence of bacteria, the abundances of tri, penta, and octa-ethylene glycol all increased, along with other compounds of unknown identity (**Figure 1A**); these results were indicative of biodegradation of K30. Relatedly, decanoate (a measured constituent of K30) was reduced to undetectable levels in the presence of *A. radioresistens* 50v1, while suberic acid levels increased; these results were suggestive of fatty acid metabolism *via* ω-oxidation. In the presence of K30, the biometabolites of 1-monopalmitin, glycerol monostearate, 4-hydroxybenzoate, and 2-ketoisocaproic acid all increased in abundance, while, in contrast, fumarate and 2-ketoglutarate decreased in abundance (**Figure 1B**). Additionally, the abundances of several alpha-hydroxy acids decreased in abundance in the presence of K30 (**Figure 1B**). Lastly, analysis of the cultivation data showed that 0.1% v/v K30 decreased the lag time by ~2-fold, while no overall impact was observed on the growth rate in the presence of 0.1% and 1.0% v/v K30.

Conclusions: Our metabolomics analyses support the bacterial degradation of K30 by the spacecraft-associated *Acientobacter*. Additionally, our work on the extracelluar metabolites is suggestive of stress responses, including impacts on iron acquisition, in the presence of K30. Hene, these results provide a potential biochemical rationale to the observed microbial ecology dynamics of spacecraft assembly facilities, where the *Acinetobacter* are among the most abundant bacteria. Future experiments will include floor-isolates from the Mars Phoenix facilities along with analysis of the intracellular biometabolites.

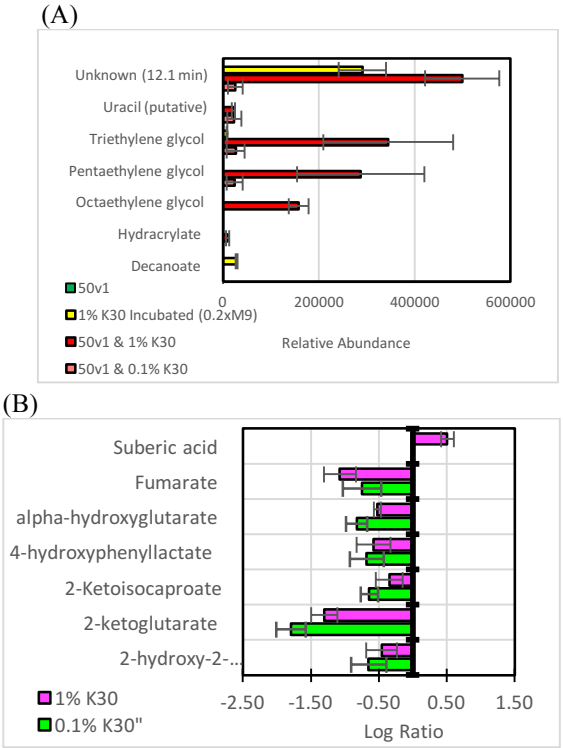

Figure 1. (A) Biodegradation of K30 and (B) change in biometabolite abundance.

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