Impact of carbon sources on the metabolomics and extremotolerance of spacecraft-associated *Acinetobacter*.

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Introduction:

We are characterizing the carbon metabolism and oligotrophic survival strategies of strains of *Acinetobacter* isolated from the assembly facilities for Mars spacecraft. As per NASA policy, the cleanliness of all Mars-destined spacecraft are carefully controlled in an effort to reduce microbial contamination and to minimize the probability of detecting false positive signals of life [1]. Our collective efforts are focused on strains of *Acinetobacter* isolated from the Mars Phoenix lander assembly facility and the surface of the pre-flight Mars Odyssey orbiter. Our prior studies have shown that all tested spacecraft-associated *Acinetobacter* possess high catalase specific activities and that *A. gyllenbergii* 2P01AA (Mars Phoenix) and *A. radioresistens* 50v1 (Mars Odyssey) display extreme tolerances towards hydrogen peroxide (H_2O_2) under nutrient rich conditions.[2, 3] Proteomic and enzymology studies on these strains suggest that the oxidative extremotolerances are related to factors associated with peroxide degradation, energy management, protein synthesis and folding, membrane transport, and nucleotide metabolism. Given the potential for these microorganisms to be forward contaminants, we herein report on our progress towards elucidating the impacts of oligotrophic conditions on the oxidative extremotolerances, carbon metabolite contents, and oxidoreductase activities of *A. radioresistens* 50v1.

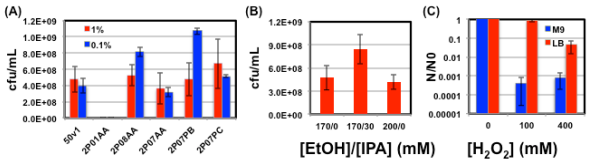
Methods:

In these studies, *A. radioresistens* 50v1 and other Phoenix-related stains were cultured under minimal conditions using M9 media supplemented with 26 µM Fe^{2+} and 0-200 mM ethanol (or mixtures of ethanol and isopropanol). Viability assays on ethanol (and alcohol mixtures) were measured by changes in optical density and by plate counts; for the plate count assays, late-log phase cultures were spread on lysogeny broth (LB) agar plates and enumerated for colony forming units (cfu/mL). Oxidative extremotolerances were measured after exposure to 100 and 400 mM H_2O_2 under both minimal and rich conditions; in short, mid-log phase cultures were exposed for 1 hr, quenched with catalase, and enumerated as described above. Measurements of catalase specific activites were performed on mid-log phase cultures, which were harvested, washed, lysed with either Bugbuster® reagent or ultrasonication, and spectrophotometrically assayed for catalase activity (240 nm) using both the supernatant and pellet components of the extract (obtained after centrifugation). Nuclear magnetic resonance (NMR) studies were performed on whole cells obtained from late-log phase cultures, which were grown in M9/Fe/EtOH or LB media, washed with PBS, resuspended in water, spiked with 1-10% D_2O, and analyzed a Varian 400 MHz NMR.

Results & Discussion:

Our results indicate that most tested strains of spacecraft-associated *Acinetobacter* grow on ethanol as a sole carbon source. Growth rates were dependent on ethanol concentration, with both plate counts and optical density measurements supporting faster growth rates in 0.1% v/v (~16 mM) ethanol (EtOH) as compared to 1% v/v (~160 mM) for *A. radioresistens* 50v1 and 3 of the 5 Phoenix-related strains (Figure 1A). While isopropanol (IPA) was not a sole carbon source under these conditions, plate counts assays supported a slow metabolism of IPA (Figure 1B). In essence, plate counts of cultures grown in 170 mM EtOH and 30 mM IPA ($8.4x10^8$ cfu/mL) were statistically significant and higher than those obtained in either 170 or 200 mM EtOH ($p<0.05$). Additional interrogations using higher mole fractions of IPA are underway.

Figures 1A-C. (A) Viability of spacecraft-associated *Acinetobacter* in 0.1 and 1.0% v/v EtOH; (B) Viability in EtOH and IPA mixtures; and (C) survival in H_2O_2.



When grown on ethanol under minimal conditions, *A. radioresistens* 50v1 displayed an extremotolerance towards H_2O_2 that was comparable to the survival under nutrient rich conditions (Figure 1C). These experiments indicated a ~3-log reduction in 400 mM H_2O_2 under minimal conditions (N_0 ~$3x10^8$ cfu/mL), whereas ~2-log reductions in survival were obtained under rich conditions (N_0 ~$5x10^8$ cfu/mL). Interestingly, the catalase specific activities were 3.4-fold higher under minimal conditions, while lysis experiments using mild detergents (Bugbuster) support a membrane-bound catalase under both minimal and rich conditions.

Our preliminary NMR analyses indicate the metabolite contents of *A. radioresistens* 50v1 are indeed dependent upon the growth conditions. Results from these experiments will be expanded upon during the presentation.

Conclusions:

Our studies indicate that the spacecraft-associated *Acinetobacter* display oxidative extremotolerances under oligotrophic conditions and (thus far) support the hypothesis that spacecraft cleaning solvents (*e.g.*, EtOH and IPA) serve as nutritional sources. Together, these findings provide a rationale towards the observed high abundances and proliferation of spacecraft-associated *Acinetobacter* in the spacecraft assembly facilities. In this presentation, therefore, we will discuss these biochemical and metabolomic trends, and the implications for planetary protection and astrobiology.

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.