Characterizing the role of denitrification in *Pseudomonas aeruginosa* biofilm formation

Nicolas L. Keshishian[1] and Boo Shan Tseng[1]
[1]University of Nevada, Las Vegas, Department of Life Sciences, Las Vegas, Nevada, 89154, United States

Whenever humans travel to space, we don't go alone. We bring trillions of microbes with us. Bacteria are found in nearly every environment and play important roles in human health. However, bacteria can also become harmful when they persist in environments where they are difficult to eliminate and acquire improved virulence. One reason for this persistence is their ability to form biofilms, which are aggregates of bacteria that attach to surfaces and surround themselves in a self-produced gel-like matrix. This biofilm helps protect bacteria from antibiotics, disinfectants, and radiation. Recent advancements in aerospace exploration have shown the need for improved biofilm containment protocols to prevent forward planetary contamination, astronaut safety, and flight longevity. Here we report one such biofilm-forming microbe, *Pseudomonas aeruginosa*, which can form biofilms in anoxic conditions. We investigated a mutant strain of *P. aeruginosa* lacking NorB, a key nitric oxide (NO) reductase enzyme responsible for reducing NO to less toxic forms. We characterized a potential role for NorB in *P. aeruginosa* biofilm formation, using a 96-well crystal violet biofilm assay, a standard method for measuring biofilm biomass. We found that the mutant strain produced significantly more biofilm than wild-type ($p < 0.01$; $N = 2$, ANOVA with post hoc Tukey HSD). This data suggests that sustained exposure to low levels of NO increased biofilm biomass. This is important because oxygen-depleted microenvironments, like that of the center of a biofilm or confined spaceflight water filtration system, have an increased likelihood of developing resistant aggregates of bacteria. Our results suggest NO metabolism may influence biofilm formation in confined environments.