

Bacterial Biofilms and Their Importance on Earth and in Space  
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Biofilms, surface-adherent microbial communities, are notorious for resistance to antimicrobial agents, fouling and corrosion, and infectious disease risks. While common on Earth, biofilms are also found on spacecraft. During spaceflight, biofilms represent risks for life-support functions and crew health. This issue is particularly critical for the Environmental Control and Life Support Systems on crewed spacecraft, such as the Water Recovery System aboard the International Space Station (ISS). As human exploration missions extend into deeper space, effective strategies for controlling biofilm formation are urgently needed. Silver-based disinfection has shown promise in long-term studies, and NASA is considering silver fluoride (AgF) for future spacecraft water systems. To assess the impact of silver disinfection on formation and persistence of biofilms, we performed the longitudinal study “Bacterial Adhesion and Corrosion” (BAC), which launched to the ISS aboard both SpaceX-21 and 29. Mixed-species biofilms of *Pseudomonas aeruginosa* and *Escherichia coli*, were developed in artificial urine medium on stainless steel and Teflon surfaces with or without 400 ppb AgF. Samples were preserved on-orbit for post-flight imaging and -omics analysis at 4 and 14 days post-inoculation, while long-duration 117-d samples were fixed upon return to Earth. Asynchronous ground controls were conducted on Earth. Unique biofilm structures, analogous to Van Gogh’s Starry Night, were seen in early spaceflight samples but not ground controls. AgF disinfection effectiveness varied between the flight and ground control samples, particularly in the context of long-term biofilm control. Only spaceflight samples exhibited viable bacterial growth in the presence of silver at the late timepoint, although there was morphological evidence of biofilm growth in both flight and ground-control samples. Ongoing genomic and transcriptomic analyses aim to provide deeper insights into biofilm dynamics, potential correlation with silver disinfection efficacy and an insight into microbial evolution during spaceflight.