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Structural analysis and resistance properties of *Bacillus subtilis* biofilms under simulated microgravity

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Starting with mission Apollo 16, the Gram positive bacterium *Bacillus subtilis* has been used in multitude of space experiments. Investigating the influence of extreme space conditions like radiation, vacuum or microgravity, experiments with model organisms like *B. subtilis*, which forms highly resistant endospores and biofilms, enlighten our understanding regarding survivability, resistance and potential virulence in unfavourable habitats. Biofilms are organized in a complex self-produced extracellular polymeric matrix commonly composed of polysaccharides, proteins and nucleic acids. Building a biofilm protects the individual cell against shear forces, chemicals (e.g. antibiotics or disinfectants), temperature changes and water as well as nutrient depletion (Vlamakis et al., 2013, Cairns et al., 2014). The intrinsic resistance of biofilms is challenging, not only in industry and medicine, but it can be problematic during spaceflight conditions, especially for the crew as well as for the spacecraft. In particular, long term missions with complex cooling systems, water supply and heat pipes may be vulnerable to biofilm colonisation.

In our work, we used a biofilm-forming *B. subtilis* strain and a biofilm-matrix deficient mutant to study the impact of reduced gravity on matured biofilms. Our major research goal is to compare biofilm formation in simulated microgravity (μg , using a fast-rotating 2D clinostat) to terrestrial gravity (1g) conditions by using different microscopic techniques. White light profilometry, scanning and transmission electron microscopy (SEM, TEM) and confocal laser scanning microscopy (CLSM) were used to analyse biofilms regarding their topology and structure, respectively. Furthermore we conducted a multitude of different survival experiments to evaluate changes and resemblances due to the impact of microgravity. First results show qualitative architectural differences between simulated microgravity and 1g in cross-sections, but no significant qualitative variations in biofilm surface topography. Biofilms grown under simulated microgravity seem to exhibit similar extreme resistances to environmental changes, compared to 1g-controls, when exposed to space-like conditions.

Keywords: biofilm, *B. subtilis*, spores, microgravity, clinostat, SEM, CLSM, profilometry

References

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