

Influence of simulated microgravity on *B. subtilis* biofilms

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Bacillus subtilis is one of the most studied Gram positive model organisms. Since mission Apollo 16, *B. subtilis* has been used for a multitude of space experiments. Investigating the influence of extreme conditions like those in space, non-domesticated strains, such as NCIB 3610 are of special interest regarding their ability to form biofilms. Since it is known that planktonic life is the exception, biofilms are considered as predominant way of living (Moons et al., 2009). 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 a problem, not only in industry and medicine, but it can be problematic under spaceflight conditions. Especially the loss of gravity coupled with changed levels of radiation might influence the resistance and therefore the virulence of bacterial biofilms. This can possibly evoke problems 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 the biofilm-forming wildtype strain NCIB 3610 and a biofilm-matrix deficient mutant (deletion of 15-gene exopolysaccharide operon, *epsA-O*) to study the impact of reduced gravity on matured biofilms. Our major research goal is to compare biofilm formation in simulated microgravity (using a 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 inner structure, respectively. First results show qualitative architectural differences between simulated microgravity and 1g in cross-sections, but no significant qualitative variations in biofilm surface topography.

Keywords: biofilm, *B. subtilis*, whole biofilm analysis, microgravity, SEM, TEM, CLSM, profilometry

References

Cairns, L. S., et al. (2014). "Biofilm formation by *Bacillus subtilis*: new insights into regulatory strategies and assembly mechanisms." Molecular Microbiology **93**(4): 587-598.

Moons P. et al. (2009). "Bacterial interactions in biofilms". Crit. Rev. Microbiol. **35**:157-168.

Vlamakis, H., et al. (2013). "Sticking together: building a biofilm the *Bacillus subtilis* way." Nat Rev Micro **11**(3): 157-168.