# Biofilm formation under simulated microgravity - a *Bacillus subtilis* case study

*Felix M. Fuchs[1,]*, Gudrun Holland[2], Ruth Hemmersbach[3], Michael Laue[2], and Ralf Moeller[1,]**

*[1]German Aerospace Center (DLR e.V.), Institute of Aerospace Medicine, Radiation Biology Department, Space Microbiology Research Group, Cologne (Köln), Germany*
*[2]Robert Koch Institute (RKI), Advanced Light and Electron Microscopy, Berlin, Germany*
*[3]German Aerospace Center (DLR e.V.), Institute of Aerospace Medicine, Gravitational Biology, Cologne (Köln), Germany*

** Corresponding authors: E-mail: felix.fuchs@dlr.de & ralf.moeller@dlr.de, Phone +49(2203) 601 -4209/-3145*

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 maturated biofilms. Our research aim 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. Different types of survival experiments were conducted to evaluate changes and resemblances due to the impact of microgravity. Our results show qualitative architectural differences between simulated microgravity and 1g in cross-sections, but no significant qualitative variations in biofilm surface topography. Our results show qualitative architectural differences in cross-sections of biofilms grown in simulated microgravity and 1g.

References :

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

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