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Biofilm formation and sporulation of *Bacillus subtilis* under simulated microgravity

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Since humans are travelling into the outer atmospheres of our planet and beyond, a new environmental habitat was established. Knowing that life - possibly since its first appearance on Earth is adapted to terrestrial gravity, the impact of microgravity on prokaryotes and humans remains mostly unclear. Investigating the influence of extreme conditions like those in space, *Bacillus subtilis* was chosen as model organism. Non-domesticated strains, such as NCIB 3610 have the ability to form biofilms as well as highly resistant endospores. Since it is known that planktonic life is the exception, biofilms are considered as predominant way of living (Moons et al., 2009). 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 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 rotating 2D-clinostat to determine the effect of simulated microgravity (sim.- μ g) and terrestrial gravity (1g) on *B. subtilis* biofilms and spores 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. In order to test the resistance of spores grown under simulated gravity, germination assays, as well as survival assays were used. First results revealed that spores grown under the influence of sim.- μ g had an increased spontaneous germination rate compared to spores grown at 1g.

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

References

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