Resistance properties and structural analysis of *Bacillus subtilis* biofilms and spores grown in simulated microgravity

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Abstract:
On Earth, the majority of bacterial life can be found in aggregates, known as biofilms. Encased within a self-build extracellular matrix, composed of polysaccharides, proteins and lipids, cells of various species live in close contact. The overall structure of biofilms offers extreme resistances towards temperature changes, shear forces, pH-changes and chemicals such as antibiotics. In the last decades, interest in biofilms has increased, due to their involvement in hard-healing or chronic wounds. In addition, biofilms are known to cause bio-corrosion and are able to clog industrial pipelines or spoil water and food.
In space, certain space facilities (i.e. MIR-space station or ISS), have been colonized by difficult to treat bacterial and fungal biofilms and spores. Only few studies investigated the influence of extra-terrestrial or space conditions on biofilms and spores and their resulting changes in structure or resistance properties (Horneck et al., 2010). We used Bacillus subtilis, a Gram-positive model bacterium and space biological model system, which produces highly resistant spores and biofilms to study the impact of simulated space conditions (e.g. microgravity, radiation, nutrient limitation) on the structure and resistance of biofilms (Fuchs et al., 2017). Our major research goal is to monitor and visualize the overall biofilms-forming process, starting with spores differentiating into biofilms in simulated microgravity (sim-µg), using a fast-rotating 2D clinostat, and terrestrial gravity (1g) conditions. White light profilometry, scanning (SEM) and transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) are then used to analyze spores and biofilms regarding their topology and structures, which are important factors for resistance properties and pathogenicity.
Young biofilms (40 h) showed architectural differences in their inner structure, when cultured under sim-µg. Cross-sections of the biofilm center and intermediate regions revealed increased matrix-production and cell phenotypes alterations in sim-µg. Surface structures (topography) and biofilm regions, which were still under development, showed no structural differences between normal gravity and sim-µg. Biofilm diameter as well as the absolute amount of cells and spores did not differ under both gravity conditions. Biofilms grown under sim-µg had a significantly higher resistance towards heavy ion bombardment. However, spores showed no morphological or resistance-dependent differences between the different gravitational conditions. These results indicate that the influence of sim-µg has an impact on structural components of biofilm, which might affect the resistance properties. In summary, sim-µg caused changes in the inner biofilm architecture and in the germination behaviour of spores, which could affect the resistance properties to radiation.

References

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