Aspergillus niger colony microstructure analysis with Scanning Electron Microscopy (SEM)

Marta Cortesao[1], Gudrun Holland[2], Michael Laue[2], Vera Meyer[3], and Ralf Moeller[1]

[1]Space Microbiology Research Group, Department of Radiation Biology, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany
[2]RobertKoch Institute (RKI), Advanced Light and Electron Microscopy, Berlin, Germany
[3]Department of Applied and Molecular Microbiology, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

Aspergillus niger, the model organism for modern biotechnology, is one of the predominant fungus detected aboard the Russian Space Station (Mir) as well as the International Space Station (ISS) [1]. The ability of fungi to colonize a variety of solid and liquid substrates in the stations indoor-closed habitat – e.g. walls, windows, life-support systems, etc. - makes monitoring fungal populations a challenge for medical and operation requirements in current and future space missions [2]. Besides, as spore formers and material biodegraders, filamentous fungi can also pose a threat to astronauts' health and spacecraft safety, in particular in long-duration missions [3]. As manned-space missions grow longer in duration and complexity, being able understand the growth of filamentous fungi aboard spacecraft - to prevent contamination as well as to produce needed drugs and food (among many other materials) [4] - is crucial for a successful future of space exploration and critically important in the maintenance of similar indoor environments here on Earth as it is the case of hospitals. The spaceflight environment of the ISS is mainly characterized by a low gravity regime, also known as microgravity. Low gravity has been shown to affect microorganisms' cellular processes, from growth rate to virulence and signaling.

To study how microgravity affects the growth of *A. niger*, a high-quality scanning electron microscopy (SEM) methodology was established at the Robert Koch Institute (RKI) in Berlin, to gain relevant information on morphological and structural changes induced by simulated microgravity on the conidiophores, spores and hyphae. This approach was adapted from previous work done with *Bacillus subtilis* biofilms [5]. For that, cultures with 5-days old colonies grown in minimum media agar at 30°C, in a filter, and were cultivated under simulated microgravity (SMG), using the Clinostat, and under normal Earth gravity (1 g) as a control. Three different *A. niger* strains were tested: wild-type (N402), melanin mutant (ΔfwnA – MA93.1), and a hyperbranching mutant (ΔracA – MA80.1). Each mutant strain had triplicate samples for each condition (SMG and 1 g) to address the possible mechanisms involved in adapting to the microgravity environment. Each colony sample had a diameter of approx. two centimeters, so three different regions of the colonies were analyzed, corresponding to different stages of development: center, intermediate and rim. Sample preparation was conducted in multiple steps - fixation, dehydration, freeze-fracture, critical point drying, and sputter-coated – before imaging at the electron microscope.

Results show a successful method to study large-dimension samples of filamentous fungi colonies, which is able to provide fine structural details, especially valuable for comparison between the mutant strains. Differences in microgravity-grown colonies were detected mainly in mycelium thickness, nevertheless, a high amount of data was retrieved and further measurements and analysis will provide additional information.

References

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