Biofilm in Space (BFS): designing a spaceflight experiment

Marta Cortesao[1], Jiaqi Luo[2], Daniel Müller[2], Zeena Nisar[3], Phil Rubin[3], Frank Mücklich[2], Ruth Hemmersbach[1], Christine E. Hellweg[1], Luis Zea[3] and Ralf Moeller[1]

[1]Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany I [2]Department of Materials Science and Engineering, Saarland University, Saarbrücken, Germany I [3]BioServe Space Technologies, University of Colorado, Boulder, USA

Introduction: Fungi in Space

The Filamentous Fungi ***Penicillium rubens*** was found on board the Russian Space Station (Mir) and the International Space Station (ISS), and poses a **threat** to spacecraft's safety and astronauts' health, especially in long duration spaceflight missions because:

- promotes material biodegradation
- has highly resistant airborne spores
- can cause respiratory diseases and hinder planetary protection
- Can form **Biofilms**

Contaminate medical materials, life-support systems, and are resistant to antibiotics!

Need for improved **study, monitoring and control of fungal biofilms!**

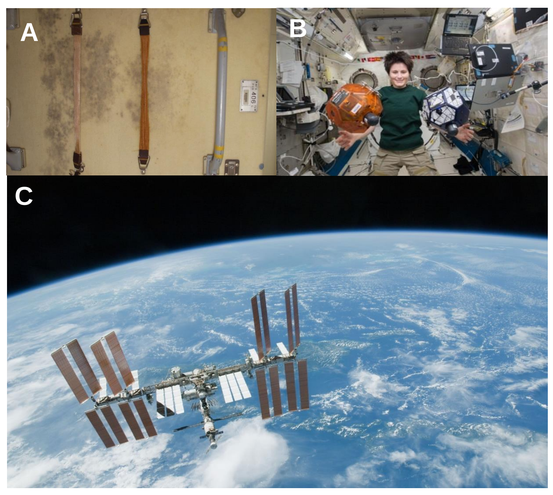


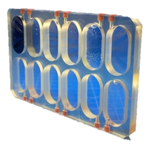
Figure 1. (A) Mold on the walls of the International Space Station (ISS) (image credit: NASA) [3]; (B) Astronaut Samantha Cristoforetti on the ISS, experiencing microgravity; and (C) The International Space Station (image credit: NASA).

This is part of a NASA-funded project with a planned space experiment aboard the ISS using BioServe's **12-well BioCell** culturing system for growth in liquid cultures under real microgravity.

ISS experiment
Real microgravity

Searching for antimicrobial surfaces:

- Are there differences in gene expression?
- How are biofilm formed?
- How different materials can influence biofilm formation?



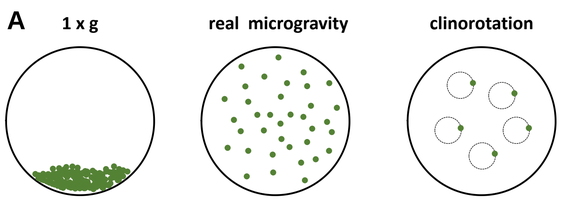
Pre-flight tests
Simulated microgravity

Setting a methodology for Fungi in the BioCell:

- How to test growth and biofilm formation, in different materials?
- What adaptations are needed compared with other containers?
- Is the 12-well BioCell an adequate culturing system for filamentous fungi in the spaceflight experiment?

Methodology

On each BioCell well: 10^5 spores/ml were inoculated on 2.3 ml of Potato Dextrose Medium with or without coupons, comparing adhered growth (on **Aluminum** and **Cellulose** membrane coupons) with planktonic growth (no coupons). The BioCells were incubated under simulated microgravity (µ x g) and normal Earth's gravity (1 x g). Samples were taken at 48h and 96h. Growth was assessed by measuring **dry biomass**. Biofilm formation was assessed by **fluorescence microscopy.**



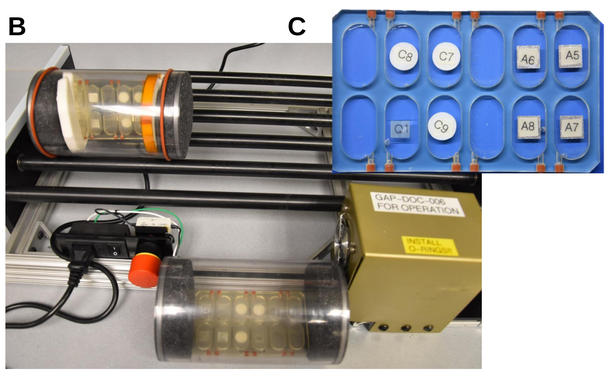


Figure 2. (A) Clinostat's principle of operation: microgravity is simulated by continuously rotating around one axis, perpendicular to the direction of the gravity vector. The continuous rotation prevents cell sedimentation, which translates to a functional simulation of the microgravity environment. (B) BioServe's Clinostat rotating the 12-well BioCells. (C) A closer look at the 12-well BioCell with Aluminum and Cellulose membrane coupons.

Results

Dry biomass

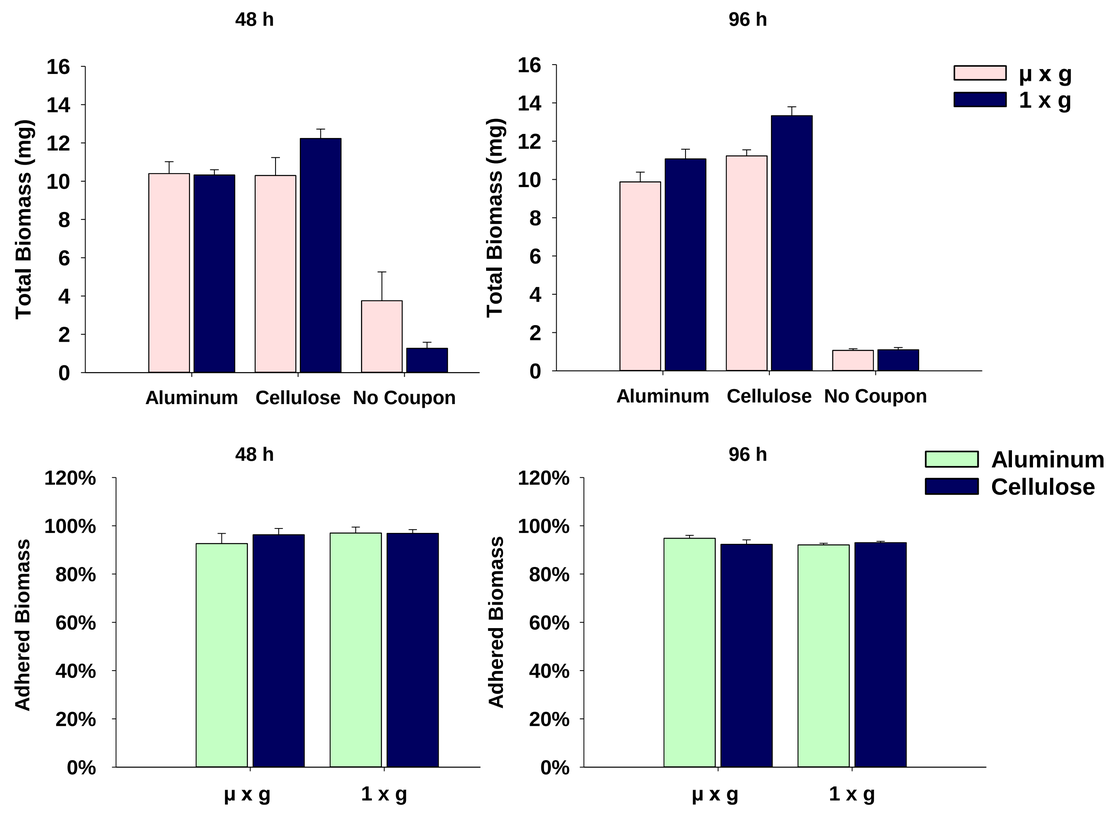


Figure 3. Dry biomass calculated as "(weight of dry filter after incubation - weight of dry filter before incubation) / weight of dry filter before incubation". On top, the total biomass produced in each well for each condition: growth with Aluminum coupons, with Cellulose membrane coupons and growth without coupons (planktonic), under simulated microgravity (pink) and normal gravity (dark blue), for 48 h and 96h of incubation. On the bottom, the percentage of adhered biomass to Aluminum (green) and to Cellulose membrane (dark blue) coupons, in both simulated microgravity (µ x g) and 1 x g, for 48 and 96h of incubation.

Biofilm formation

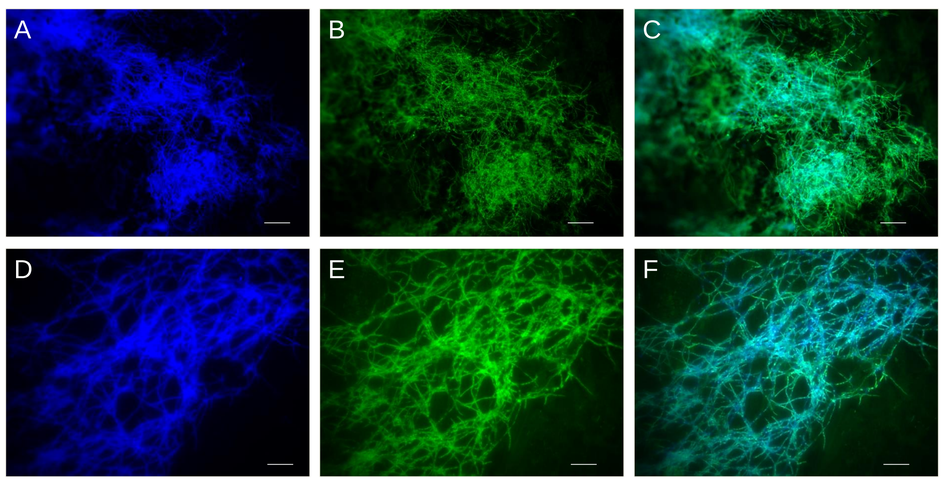


Figure 4. Fluorescence microscopy pictures showing *P. rubens* biofilm attached to Aluminum coupons exposed to simulated microgravity in the BioCells (Top: A-C) and to normal gravity at the bench (Bottom: D-F) after 96h of incubation. Staining was done with 10 µL of 0.1% CFW plus 10 µL of 1 mg l-1 AO and waiting 15 minutes in the dark before visualization. Calcofluor white (dark blue) stains chitin, a cell wall component, revealing the hyphal structure; Acridine Orange (green) stains double stranded DNA – mainly extracellular DNA - revealing the overall biofilm matrix. Light blue areas represent overlap of chitin regions and DNA within the hyphae/spores. Scale: 20 µm.

Summary

- 48h is enough to assess *P. rubens* biofilm formation, in the tested conditions
- The BioCell is an adequate culturing system for filamentous fungi
- Important step in novel methodologies to study filamentous fungi

References

1. Checinska, A. et al. Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. Microbiome 3, 50 (2015).
2. Alekhova, T. a. et al. Monitoring of microbial degraders in manned space stations. Appl. Biochem. Microbiol. 41, 382–389 (2005).
3. Novikova, N. et al. Survey of environmental biocontamination on board the International Space Station. Res. Microbiol. 157, 5–12 (2006).
4. Gomoiu, I., Chatzitheodoridis, E., Vadrucci, S., Walther, I. & Cojoc, R. Fungal Spores Viability on the International Space Station. Orig. Life Evol. Biosph. 46, 403–418 (2016).
5. Harding MW. et. al. Can filamentous fungi form biofilms? Trends Microbiol. 11, 475-80 (2009).
6. Herranz R. et al. Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology. Astrobiology. 13 (1) (2013).
7. Zea, L., et al. Design of a spaceflight biofilm experiment. Acta Astronautica 148 (294-300) (2018).

Acknowledgements

This material is based upon work supported by the National Aeronautics and Space Administration under Grant No. 80NSSC17K0036. The work performed in the Dr. Moeller laboratory was supported by a grant from the German Aerospace Center (DLR grant: DLR-FuW-Projekt ISS LIFE, Programm RF-FuW, Teilprogramm 475). Marta Cortesão's doctoral thesis is funded by DAAD - Deutscher Akademischer Austauschdienst,

Contact

Marta Cortesão
PhD Student
marta.cortesao@dlr.de
+49 2203 605 4211

www.spacemicrobes.com

facebook.com/spacemicrobes
instagram.com/spacemicrobes