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Life and Microgravity Sciences on board ISS and beyond (Part II) (7)

Author: Ms. Marta Cortesao  
German Aerospace Center (DLR), Germany

Mr. Philip Rubin  
University of Colorado Boulder, United States

Mr. Jiaqi Luo  
Saarland University, Germany

Dr. Christine Hellweg  
German Aerospace Center (DLR), Germany

Dr. Louis Stodieck  
University of Colorado Boulder, United States

Prof. Frank Mücklich  
Saarland University, Germany

Prof. David Klaus  
University of Colorado Boulder, United States

Dr. Ralf Moeller  
German Aerospace Center (DLR), Germany

Dr. Luis Zea  
University of Colorado Boulder, United States

CONTROLLING SPACEFLIGHT FUNGAL BIOFILMS: THE SEARCH FOR ANTIMICROBIAL  
SURFACES**Abstract**

Fungal biofilms have been detected on board the Russian Mir Space Station and the International Space Station (ISS), posing a concern to human health and to spacecraft integrity. To improve monitoring and control of fungal contamination, a NASA-funded spaceflight experiment is currently being designed to study bacterial and fungal biofilms while also testing for antimicrobial surfaces. To inform the spaceflight experimental design a series of ground tests were conducted on BioServe's 12-Well BioCell, defining and optimizing the culturing conditions, in this case, for the fungus *Penicillium rubens* (formerly *Penicillium chrysogenum*).

Because growth in the BioCell will inevitably differ from common laboratory containers (such as flasks or multi-well plates), it is important to assess i) if the fungus can grow and form biofilms; and ii) if there is adherence to coupons and how it is compared with planktonic growth. For that an initial approach tested *P. rubens* growth in the 12-well BioCell, both in simulated microgravity provided by clinorotation ( $\times g$ ) and in ground static control ( $1 \times g$ ), as well as its adherence to two different material coupons - cellulose membrane and aluminum. Total, adhered and planktonic biomass was measured in each well, and fluorescence microscopy of coupon attached biomass was used to identify the presence of hyphae and surrounding matrix of the fungal biofilm.

Statistical analysis show no significant difference between the two time points of 48h and 96h, suggesting that an incubation time of 48h is enough for this setting. Differences in total biomass between simulated  $\times g$  and  $1 \times g$  are significantly different, showing cellulose membrane coupons to have 26% more total biomass than aluminum coupons in  $\times g$  and 19% more total biomass in  $1 \times g$  conditions. This

overall increased growth under simulated microgravity may be due to the mixing of the media which may have led to extensive surface contact and consequently increased oxygen availability. Cellulose membrane was also shown to have more adhered biomass than aluminum coupons, as expected, with 19% and 28% more adhered biomass in both  $\times g$  and  $1 \times g$  conditions, respectively.

This experiment established the 12-well BioCell as an adequate culturing system for growth of *P. rubens* in the upcoming spaceflight experiment. Most importantly, this marks an important step in the study of filamentous fungi biofilms, both on Earth and in space.