Growth and biofilm formation of *Penicillium chrysogenum* in simulated microgravity

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Introduction: Fungi in Space

Penicillium* and *Aspergillus are two of the main fungal genera detected on board the Russian Space Station (MIR) and the International Space Station (ISS) [1-3]. On Earth these fungi are used for biotechnological applications, producing antibiotics such as penicillin, and other relevant compounds like citric acid. The use of fungi in space applications, (microbiology, medicine, biotechnology) is an opportunity. However, fungi can also pose a **threat** to spacecraft's safety and astronauts' health [4] because:

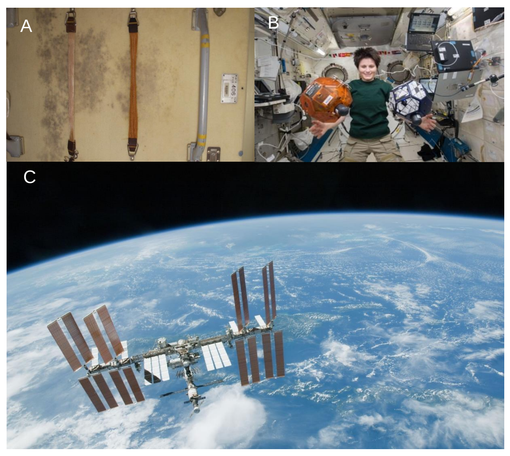

Figure 1. (A) Mold on the walls of the International Space Station (ISS) (image credit: NASA) [3]; (B) Astronaut Samanta Cristoforetti on the ISS, experiencing microgravity; and (C) The International Space Station (image credit: NASA).

- They promote material biodegradation
- The highly resistant airborne spores can cause respiratory diseases and hinder planetary protection
- Can form **Biofilms** ➡ Contaminate medical material and water systems = promote resistance to antibiotics and adverse conditions!

⬇

Threat to long duration spaceflight missions!

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Aspergillus fumigatus is the most studied. Yet filamentous fungal biofilms are not well understood!

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Need for improved **study, monitoring and control of biofilms, both on Earth and in Spaceflight**

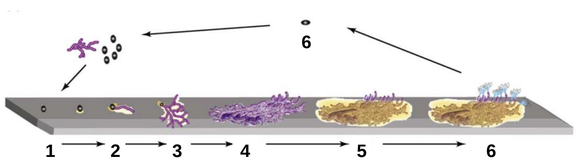


Figure 2. Model for filamentous fungi biofilm development, based on what is known from bacteria and yeast. Modified from Harding et al. 2009 [5]. A biofilm can be roughly characterized as: different types of cells, embedded in a self-synthetized extracellular polymeric matrix, attached to a substratum or to themselves.

The project and pre-flight testing

NASA-funded project

This is part of a NASA-funded project with a planned space experiment aboard the ISS. This poster presents part of the initial pre-flight tests done to prepare the ISS experiment.

The ISS experiment

Grow microorganisms in BioServe's 12-well **BioCell** culturing system for growth in liquid cultures under microgravity. The study will:

- Assess differences in gene expression
- Characterize biofilm formation
- Study how coupons of different materials can influence biofilm formation

Pre-flight tests

The filamentous fungus ***Penicillium chrysogenum* DSM 1075** pre-flight tests include:

- Optimizing launch conditions: media and temperature (optimal at 4° C)
- Assess growth and morphology under simulated microgravity
- Evaluate biofilm formation in vitro: culturing conditions and analysis

Methodology

Penicillium chrysogenum spores suspended in 0.9 % NaCL were inoculated at the very center of petri dishes and triplicates were incubated under different conditions: **media, temperature and simulated microgravity**. Growth, colony morphology and biofilm formation were assessed qualitatively when incubated in Potato Dextrose Agar or Broth, at room temperature of 22° C (similar to ISS). **Microgravity** was simulated by continuously rotating the **BioCell** (Fig. 3B) around one axis perpendicular to the direction of the gravity vector using a 2-D Clinostat (Fig. 3C).

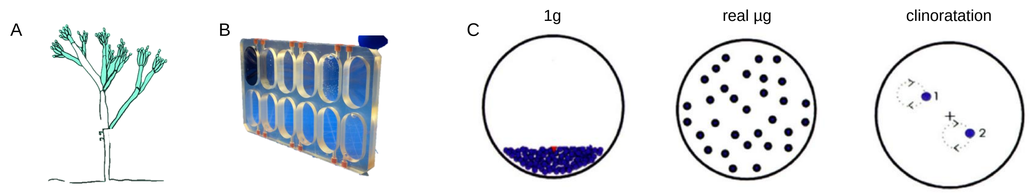


Figure 3. A) Drawing depicting *Penicillium chrysogenum*. B) The 12-well BioCell. C) Schematic illustrating a clinostat's principle of operation [6]. The continuous rotation prevents cell sedimentation, which translates to a functional simulation of the microgravity environment.

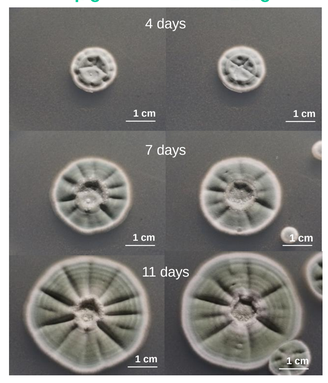
Pre-flight testing: preliminary results

A. Testing launch conditions

3 weeks
RT ~ 22°C
4°C
Media

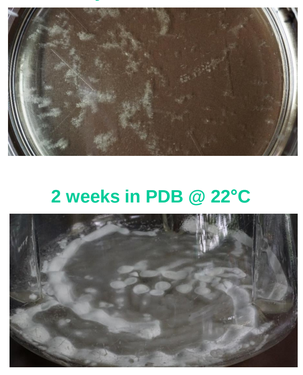
B. Colony morphology under simulated microgravity

µ g
1 x g



C. Biofilm formation in vitro

2 days in PDB @ 22°C



2 weeks in PDB @ 22°C

Figure 4. 10^6 spores/ml incubated in different media (PDA = Potato Dextrose Agar. CM = Complete medium; MM = Minimum medium) and different temperatures (22ºC and 4° C) for 3 weeks to test launch conditions (optimal at 4° C). All present grow at 4° C, which is undesirable. PDA is the preffered media for morphological characterization and is the preffered media for culturing conditions. B) Incubation of 10^6 spores/ml in PDA media both at normal gravity (1 x g) and simulated microgravity (µg) using a 2-D petri-dish clinostat, for 11 days at room temperature (22ºC). C) Assessing biofilm formation in vitro by inoculating 10^5 spores/ml suspended in 0.9 % NaCL in PDB = Potato Dextrose Broth at room temperature (~22ºC) Static, for 2 days and for 2 weeks.

Summary and Future work

Summary

- ✓ The ISS experiment has optimal launch conditions at 4ªC however, *Penicillium chrysogenum* is able to grow at 4° C in all tested media
- ✓ Tested simulated microgravity causes no observable changes in morphology

Future work

- ✓ Growth in the BioCell with the different coupons, in 1 x g and µg
- ✓ Biofilm analysis: biomass, morphology, fluorescence microscopy
- ✓ Optimize launch conditions according to space requirements

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