Evolutionary Stability of Microbial Mutualism in Simulated Microgravity

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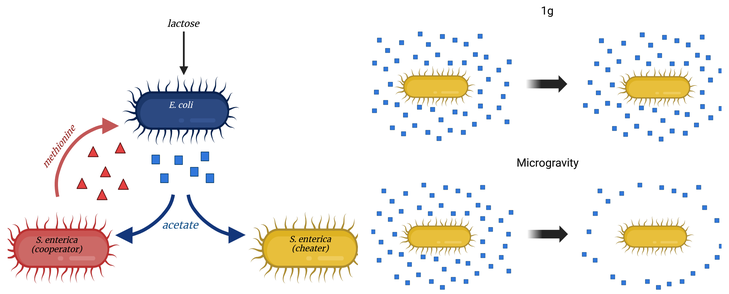
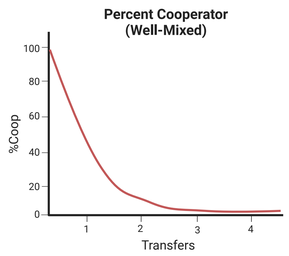
INTRODUCTION

In this study, we investigated the evolutionary stability of mutualistic interactions in multispecies communities and the robustness of those communities in microgravity using a well-studied model system of metabolite transfer.

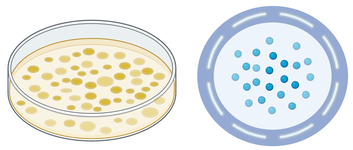


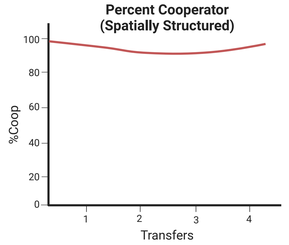
In 1g, convective mixing and sedimentation circulates metabolites and microbes throughout the system, providing the microbes access to metabolite from the entirety of the system. Suspension of microbes in microgravity, reduces these effects and creates spatial structure. Within a spatially structured environment, metabolite transfer is diffusion-limited and "zones of depletion" [1] are created by suspended microbes consuming all metabolites in their vicinity. These conditions make microbes reliant on their immediate neighbors.

Non-Spatially Structured (Shake Flask)



Spatially Structured (Petri Dish and Spin Pod)





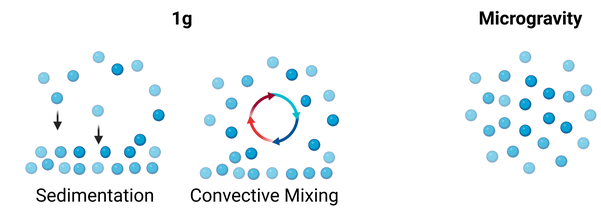
Previous studies using the same microbial system in spatially structured (solid) cultures vs. non-spatially structured (shaken liquid) cultures showed mutualism to be favored in spatially structured environments. [3]

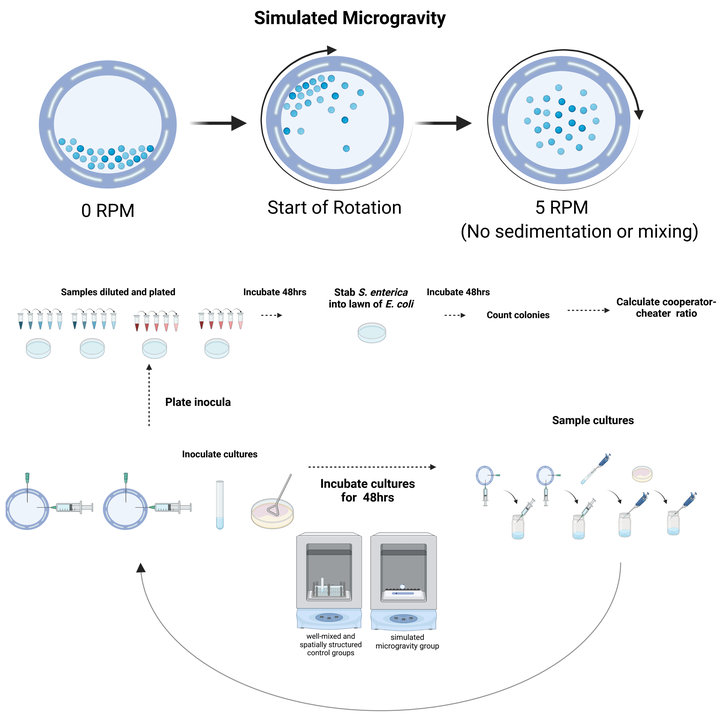
We hypothesized the spatial structure created by microgravity would behave similar to that of petri dish cultures and favor a stable population of cooperator S. enterica in non-motile strains. However motile strains should be able to resist the effects of microgravity, escape their zones of depletion, and show growth similar to communities in non-spatially structured environments.

SIGNIFICANCE

Sustainable long-term space travel will require astronauts to live in built environments cohabited by microbial communities. Though much of space biology research has focused on monocultures of microbes, microbes naturally live and interact mutualistically in multispecies communities, many of which can be harnessed as tools for biotechnology with practical applications in space[2]. Understanding the effects of microgravity on microbial systems can inform the design of engineered microbial systems for mission-critical tasks in future manned missions.

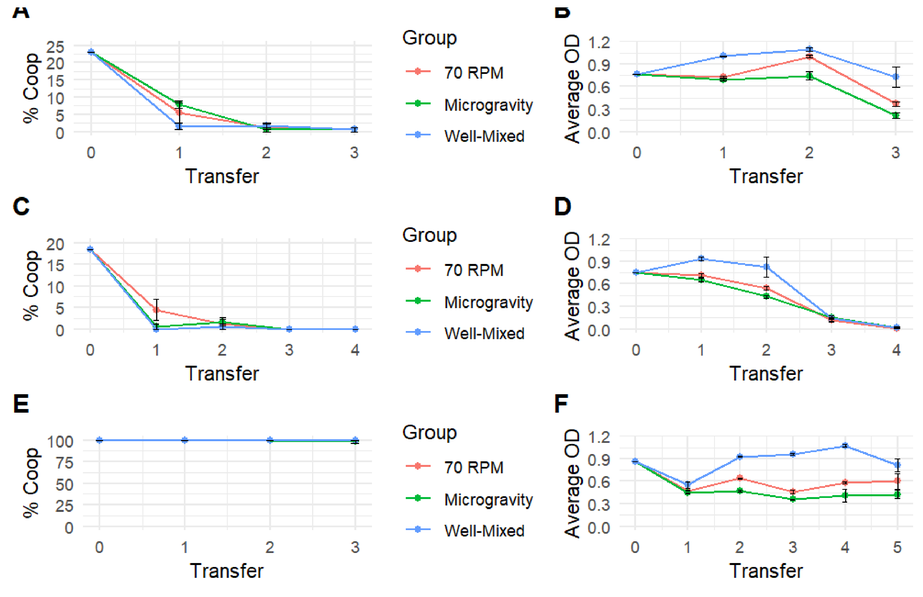
METHODS





Our model community was cultured for approximately 20 generations in simulated microgravity. Additional cultures were grown on agar plates and in shaken flasks as non-structured and structured controls, respectively. After culturing, we measured the cooperator/cheater ratios of these populations by stabbing colonies of *S. enterica* from samples into plates with *E. coli* and X-gal. Cooperators become blue and cheaters do not change color.

DATA



A) Percentage of cooperators in *Salmonella enterica* cultures with 50% cheaters and 50% cooperators across 3 transfers. B) Optical density (OD) of cooperating cultures inoculated with 50% cheaters and 50% cooperators across 3 transfers (48 hours between transfers). C) Percentage of cooperators in *S. enterica* cultures with 1% cheaters and 99% cooperators across 4 transfers. D) Optical density of cooperating cultures inoculated with 1% cheaters and 99% cooperators across 4 transfers. E) Percentage of cooperators in *S. enterica* cultures inoculated with 100% cooperators across 3 transfers . F) Optical density of cooperating cultures inoculated with 100% cooperators across 5 transfers.

DISCUSSION

All data shown are from motile strains. In the absence of cheaters, optical density remained stable for ~20 generations. Well-mixed cultures showed higher growth than simulated microgravity, with RPM 70 growth being intermediate. This trend was consistent across all cooperator-to-cheater ratios. In cultures with cheaters, rapid growth was followed by a sharp decline in OD after two transfers, indicating out-competition of cooperators. This was reflected in the cooperator percentages, which dropped sharply in 50/50 inoculations, reaching 0% by the end. Interestingly, cooperators declined more slowly in simulated microgravity than in well-mixed and RPM 70 conditions. This pattern was not observed in the 99/1 cooperator-to-cheater ratio.

Motile strains likely escaped depletion zones, showing growth and cooperator/cheater dynamics similar to those in non-spatially structured environments. Future experiments will use non-motile strains to further explore this behavior.

REFERENCES

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