Microbial communities in microgravity: simulation in lab and on the computer

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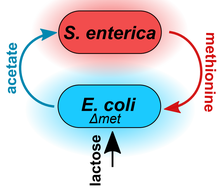
Microbial communities come with us to space

Multispecies microbial communities are important in our microbiomes, and in applications like waste processing and bioproduction.
But space biology research has usually focused on individual species.

A model community of cross-feeding bacteria demonstrates that mixing is important to cooperation

We work with a model cross-feeding community, where each species is dependent on a compound the other produces [1,2].

Prior work has shown that on **short (ecological) timescales**, community growth rate depends on the rate of substrate exchange, so it is faster in well-mixed environments.



On **long (evolutionary) timescales**, cooperation is stable in slow-mixing environments because benefits of cooperation are localized, but unstable in well-mixed environments, because cheaters win when benefits are globally distributed.

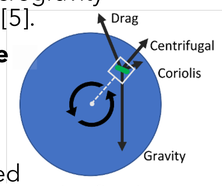
Microbes experience microgravity through slower mixing

Without gravity, there is no density-driven convection: all mixing is through diffusion [3].

This may result in substrate depletion and waste accumulation near cells.

Microbial responses to spaceflight are consistent with starvation and acid stress [4].

Rotating wall vessels (RWVs) attempt to reproduce the quiescent microgravity environment [5].

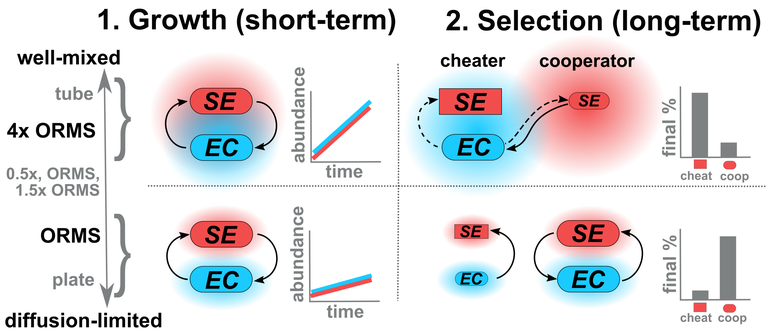


Rotation rate is key to balancing forces so that each cell is confined to a small zone in the fluid mass, and mixing is minimal.

What about microbial communities in microgravity?

Slower, diffusion-based substrate exchange in microgravity should result in:
1. slower growth by cooperative cross-feeding communities
2. less success for cooperators, more success for cheaters

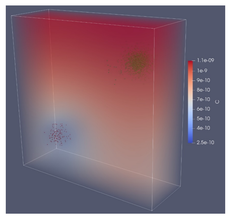
Our hypothesis: rotation rate determines ability of the RWV to simulate the quiescent microgravity environment, and therefore determines community behavior on short and long timescales



ORMS = Optimal Rotation rate for Microgravity Simulation

On computer: Computational models can predict how microgravity affects substrate concentrations around microbes

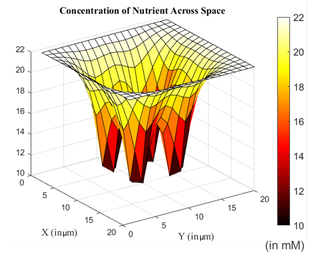
CAMDLES: when RWV matches microgravity, and when it doesn't



Model simulation of ***E. coli*** (green) and ***S. enterica*** (red) colonies spaced distantly in microgravity; color scale represents acetate concentrations

CFD-DEM Simulation of Microbial Communities in Spaceflight and Artificial Microgravity simulates fluid and particle dynamics and simplified microbial metabolism and growth. Whether RWV successfully simulates microgravity depends on spatial distribution of cells, biofilm formation, and product yield. **Read more at An & Lee (2022) Life [6].**

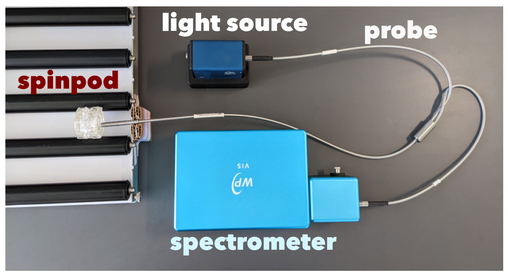
How big are substrate depletion zones in microgravity, anyway?



A model combining Finite Difference Method diffusion calculations and Michaelis-Menten substrate uptake calculations predicts the size of substrate depletion zones around bacterial cells in the diffusion-limited environment of microgravity. **Read more at Juliana Gesztesi's poster UH30 on Saturday**.

In the lab: A fluorescence system enables monitoring of 2-species growth during rotation in a RWV

To validate our computational simulations and test our hypotheses, we need to measure growth rate. Most RWV experiments require destructive sampling, making growth rate measurements difficult.
I am building **an experimental system to measure growth of both species in a RWV in real time**.
It uses optically clear rotating wall vessels (Cell Spinpods) and a visible-light spectrometer with backscatter probe.
Each species has a distinct constitutive fluorescent label: *E. coli* = Cyan Fluorescent Protein, *S. enterica* = Yellow Fluorescent Protein.

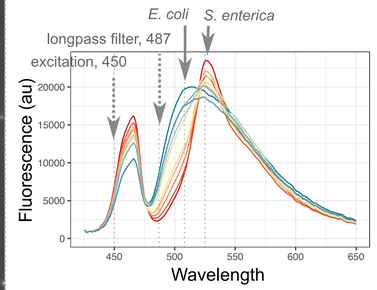


closeup of spinpod and probe

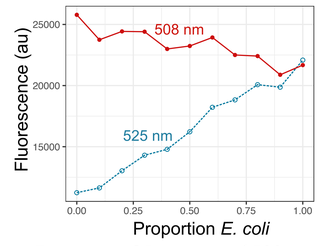
References: [1] Harcombe WR. (2010) doi:10.1111/j.1558-5646.2010.00959.x **[2]** Harcombe WR, et al. (2014). doi:10.1016/j.celrep.2014.03.070 **[3]** Klaus D, et al. (1997) doi:10.1099/00221287-143-2-449 **[4]** Aunins TR, et al. (2018). doi:10.3389/fmicb.2018.00310 **[5]** Hammond TG & Hammond JM. (2001) doi:10.1152/ajprenal.2001.281.1.F12 **[6]** An R & Lee JA. (2022) doi: 10.3390/life12050660
Acknowledgements: We thank Willian Harcombe and Jeremy Chacon for access to the model cross-feeding community and related data. Funding for this work was provided by the NASA Space Biology Program, Early Career Grant proposal 20-FG20_2-0015, and support for R. An and J. Gesztesi was provided by the NASA Space Life Sciences Training Program

Laboratory model results (so far)

Fluorescence can distinguish *E. coli* from *S. enterica* at a single illumination wavelength

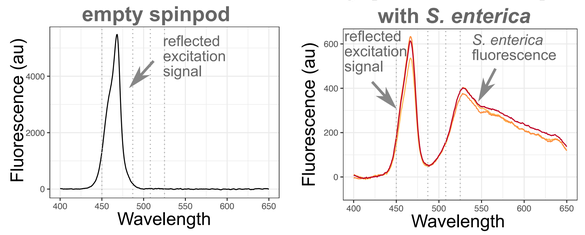


Spectra from mixed cultures of *S. enterica* (YFP) and *E. coli* (CFP) at a range of species ratios, in a cuvette. OD is constant for all mixes. Excitation: 450 nm.



For a given OD, heights of 508 nm peak (*S. enterica*) and 525 nm peak (*E. coli*) correlate to species ratio.

S. enterica can be detected by probe in a spinpod



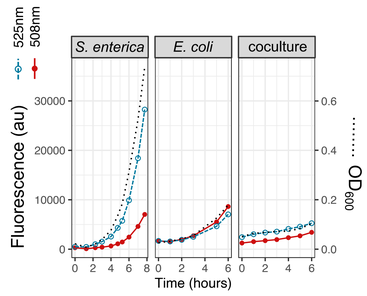
Spectrum from Cell Spinpod with sterile medium (left) and 3 spectra from a Cell Spinpod with dense *S. enterica* (YFP) culture (right), illuminated at 450 nm using backscatter probe 2 mm from surface of spinpod. Integration time 4 seconds. Note different scales on y-axis.

Challenges

Only one probe. Replicates need to be run in series.
Signal from excitation LED interferes with fluorescence signal. Plastic, medium, and cells reflect the excitation wavelength, and bandpass filters do not block it perfectly. Depends partly on incidence angle. Improving fluorescence detection requires optimization.
Fluorescence signal from spinpod is not yet quantative. Signal strength depends on distance from sample and other difficult-to-control variables. Quantitation will require fancier hardware or developing a built-in control.

Next steps

Growth curves of single and paired cultures in RWVs at varying rotation rates.
Test hypothesis 1: co-culture grows slowest at rotation rate most closely simulating microgravity.
Test hypothesis 2: cooperator strain of *S. enterica* wins over cheater at rotation rate most closely simulating microgravity.
Transcriptomic sequencing to measure gene expression changes
Simulated space radiation: Another component of this study! Ask me for more detail.



Growth curves of *S. enterica* (left) and *E. coli* (center) grown separately, and (right) as a cross-feeding coculture in lactose minimal medium, in shaking culture flask. Fluorescence spectra and OD measured in cuvettes.