University of Dundee

Founder cell locations predict outcome of competitive interactions within colony biofilms

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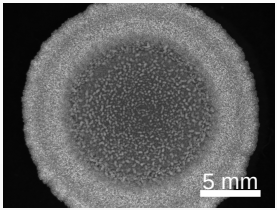
Lukas Eigentler

Biofilms

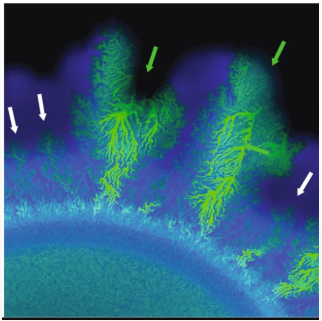
- Bacterial biofilms are surface-adhering multicellular collectives embedded in a self-produced extracellular matrix.
- Biofilms can have both beneficial and detrimental effects on the surrounding environment.
- A range of in vitro methods have been developed to study biofilms, for example the colony biofilm model.



Colony biofilm model

- Method: founding cells are deposited on an agar-solidified growth medium; after incubation, the macroscale structure is examined.

- Widely used, for example:
- Cross feeding between variants of *Pseudomonas stutzeri* induces fractal-like patterns.[1]



[1] Goldschmidt, F. et al.: *ISME J.* (2021)

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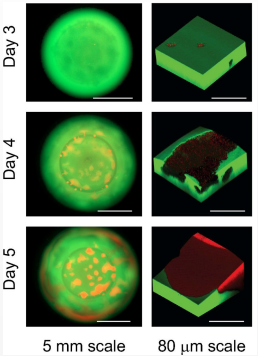

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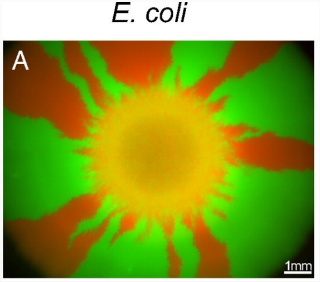
- Genetic drift induces spatial segregation.[3]



[1] Goldschmidt, F. et al.: *ISME J.* (2021)
[2] Kim, W. et al.: *PNAS* 111.16 (2014)
[3] Hallatschek, O. et al.: *PNAS* 104.50 (2007)

Competition within biofilms

- Different strains/species compete within biofilms.
- Example: the soil-dwelling bacterium *Bacillus subtilis* forms biofilms on the roots of plants, where some strains promote the growth of plants.
- To fully realise their potential as biocontrol agents, strains need to be capable of coexisting with (or outcompeting) other biofilm-forming strains in the rhizosphere.
- Many mechanisms of competition require spatial co-location of strains.
- Take a step back: need to understand the role of spatial structure first.

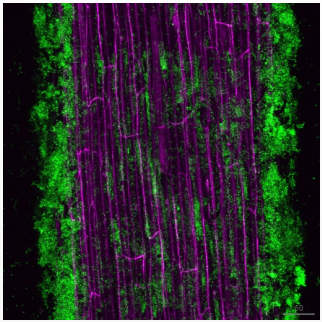
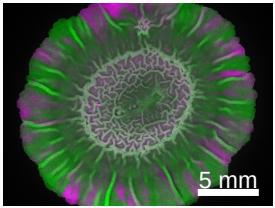


Figure by Emma Bissett

Competition within biofilms

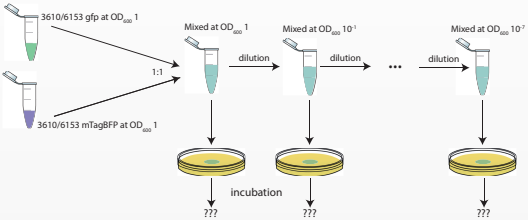
- Spatial structure is best studied using isogenic strains: all other competitive mechanisms (e.g. kin discrimination) are excluded from the model system by design.
- Isogenic strains: Low founder densities promote spatial segregation and formation of spatial sectors.[1,2]
- Questions: How does spatial structure arise and how does it affect competitive interactions?



[1] van Gestel, J. et al.: *ISME J.* 8.10 (2014)
[2] Martinez-Garcia, R. et al.: *PLOS Comput. Biol.* 14.4 (2018)

Methods

Experimental assay:



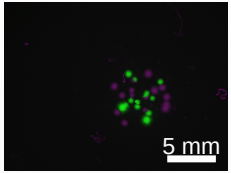
Mathematical model for isogenic strain pair:
change in time = spatial spread + growth

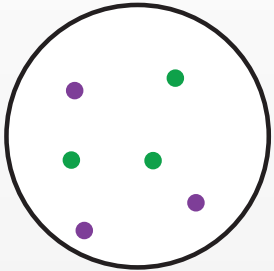
$$\frac{\partial B_1}{\partial t} = \nabla \cdot \left(\left(1 - (B_1 + B_2)\right) \nabla B_1 \right) + B_1 \left(1 - (B_1 + B_2)\right),$$
$$\frac{\partial B_2}{\partial t} = \nabla \cdot \left(\left(1 - (B_1 + B_2)\right) \nabla B_2 \right) + B_2 \left(1 - (B_1 + B_2)\right).$$

- What are appropriate initial conditions?

Initial conditions

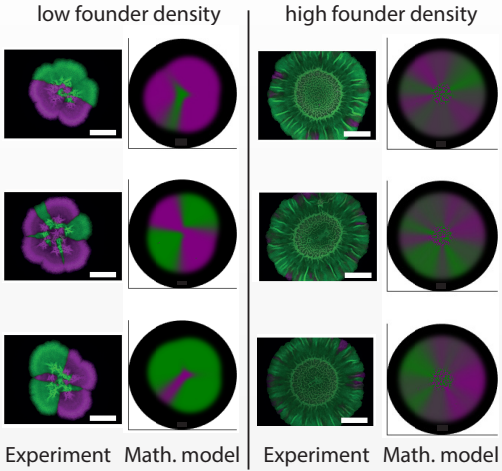
- In experiments, cells settle at random locations within the initial spot and grow to small micro-colonies.
- In the model, we position initial "cell patches" at random locations in the domain centre.
- Each model patch represents 1 microcolony $\Rightarrow$ tool to modulate founder density.





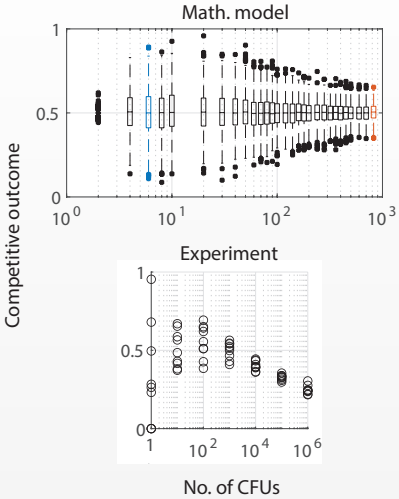
Variability in competitive outcome

- High founder density: no spatial structure and initial strain ratio consistently determines competitive outcome.
- Low founder density: spatial segregation occurs. Large variability in competitive outcome for fixed initial strain ratio.
- Founder density significantly affects phenotype and variability in competitive outcome.



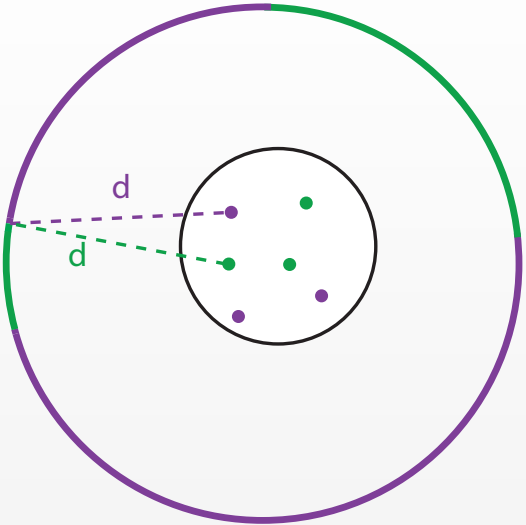
Variability in competitive outcome

- Founder density significantly affects phenotype and variability in competitive outcome.
- Variability increases with decreasing founder density.
- Note the computational power of the mathematical model: 1000 model simulations each vs 12 technical replicates each of experimental assay.



Disentangling variability

- Hypothesis: only initial patches that can drive the biofilm's radial expansion contribute to outcome density.
- We define a quantity that, based on the initial cell locations, measures a strain's "access to free space"



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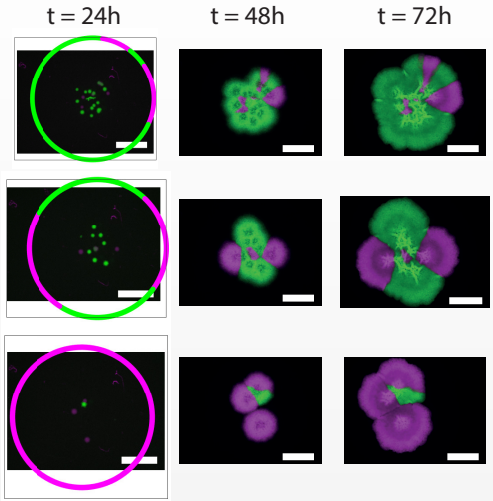
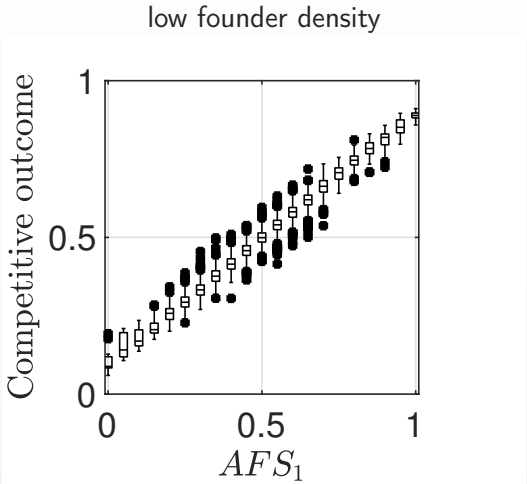


Access to free space predicts outcome

- Access to free space determines competitive outcome in the absence of any other competitive dynamics (isogenic strains).



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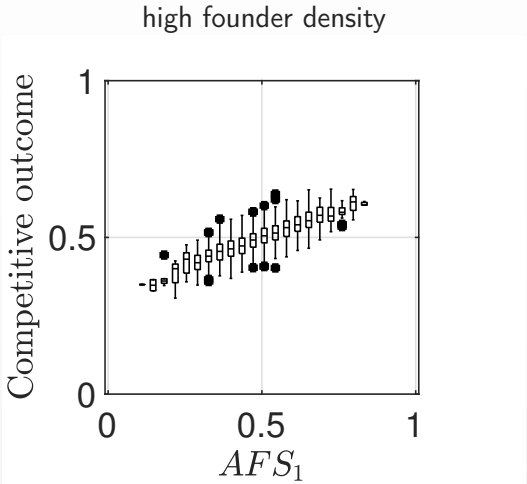
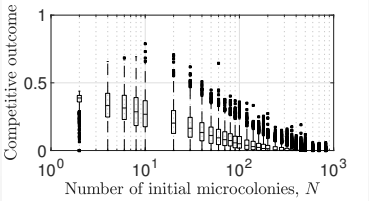
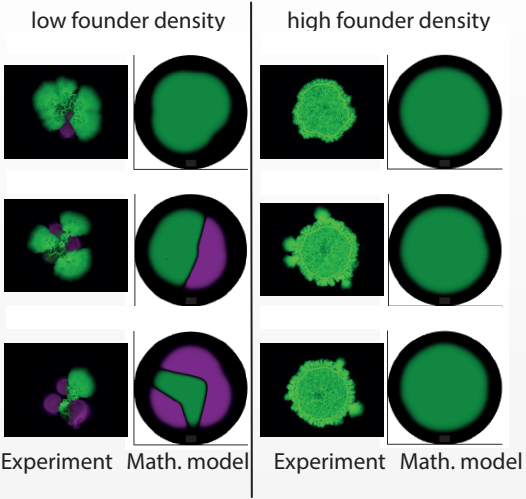
- Slope of relation between access to free space and competitive outcome depends on founder density.



Non-isogenic strains

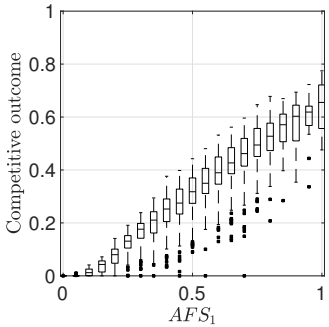
- High founder density: competitive exclusion.
- Low founder density: spatial segregation enables coexistence.
- Decreases in founder density cause (i) increased variability in competitive outcome, (ii) higher (on average) densities of weaker strain.





Access to free space predicts outcome

- Access to free space remains a reliable predictor of competitive outcome for low founder densities.



Conclusions

- Large variability in competitive outcome occurs for biofilms inoculated at low founder density.
- We revealed that this variability is induced by the random positions of founder cells within the inoculum.
- Competitive outcome can be predicted based on founder cell locations.
- Predictions hold true even if killing between strains occurs $\Rightarrow$ "Race for space" is more important than antagonistic actions at low founder densities.
- Impact on applications (e.g. use of *B. subtilis* as biofertilizer): Competitive success across all founder densities can only be guaranteed if a strain spreads fast and kills efficiently.

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