University of Dundee

Founder cell locations predict outcome of competitive interactions within colony biofilms

Stochastic Models and Experiments in Ecology and Biology

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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.
- 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.


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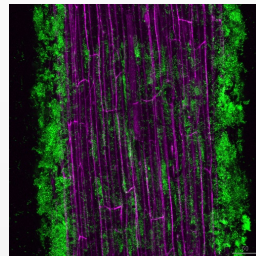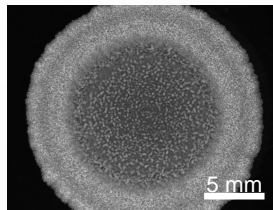
Competition within biofilms

- Competition in biofilms is underpinned by kin discrimination.
- Many mechanisms of kin discrimination require spatial co-location of strains.
- Take a step back: need to understand the role of spatial structure first.
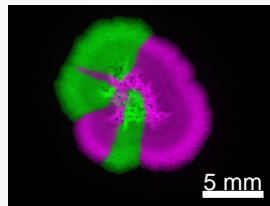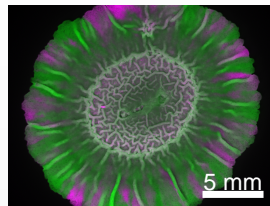
Competition within biofilms

- 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]

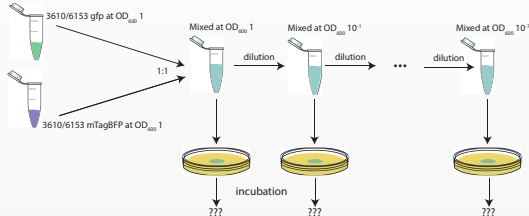- Questions: How does spatial structure arise and how does it affect competitive interactions?

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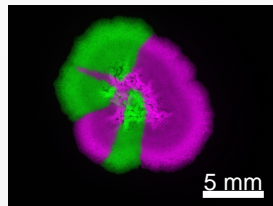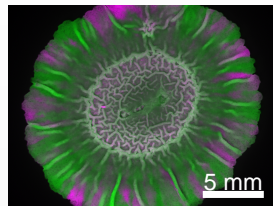
[1]van Gestel, J. et al.: *ISME J.* 8.10 (2014)
[2]Martinez-Garcia, R. et al.: *PLOS Comput. Biol.* 14.4 (2018)

Methods

Mathematical model for isogenic strain pair:

Experimental assay:

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

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

- Circular domain
  $\Omega = \{x \in \mathbb{R}^2 : \|x\| \leq R_\Omega\}$.
- 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 ⇒ tool to modulate founder density.


5 mm
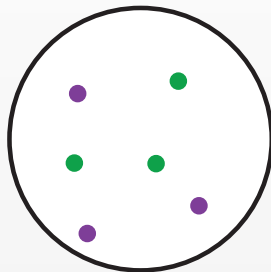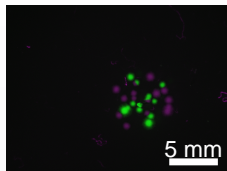
Variability in competitive outcome

- 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.

low founder density   high founder density

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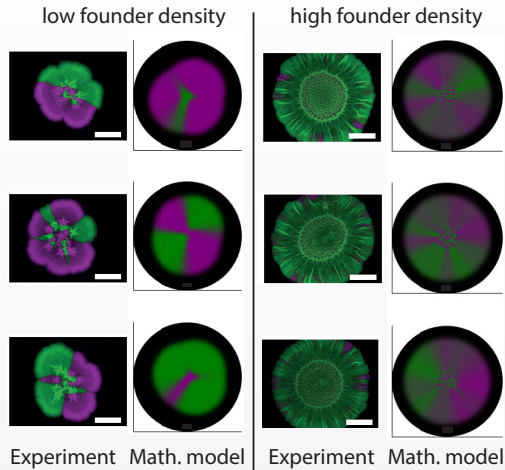
- Founder density significantly affects phenotype and variability in competitive outcome.
- Variability increases with decreasing founder density.
- Note the computational power of the mathematical model: 1000 model simulations each vs 12 technical replicates each of experimental assay.
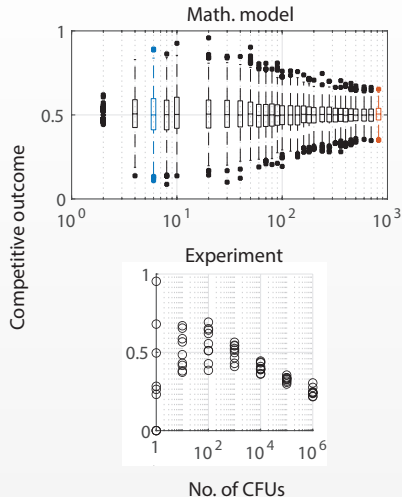
Math. model

Experiment

Competitive outcome

No. of CFUs

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"
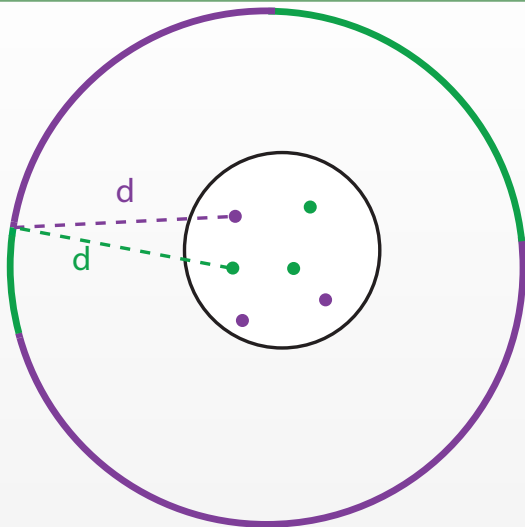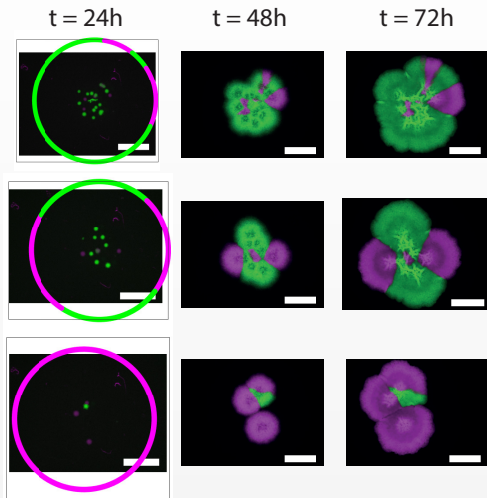
Disentangling variability

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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).

low founder density
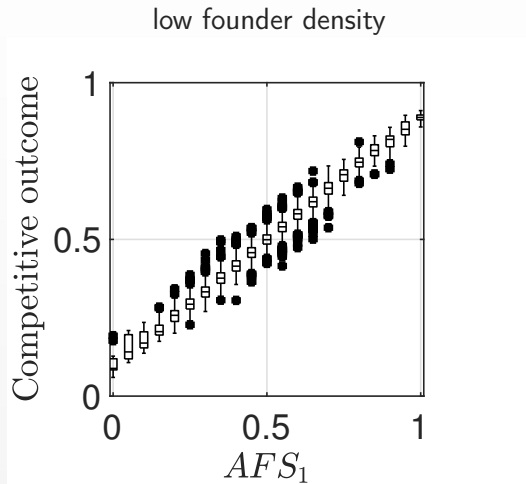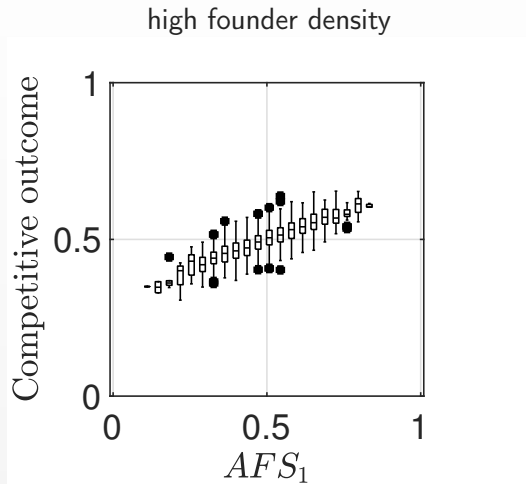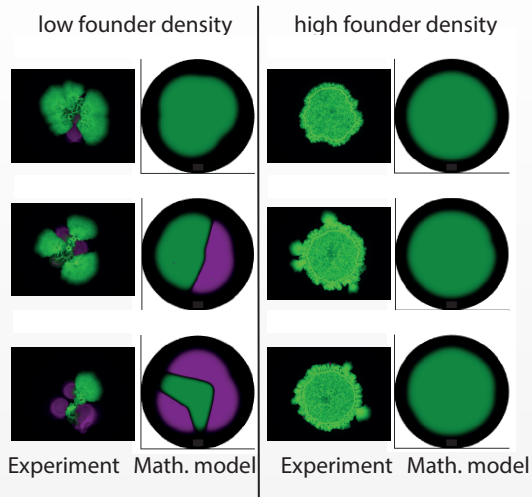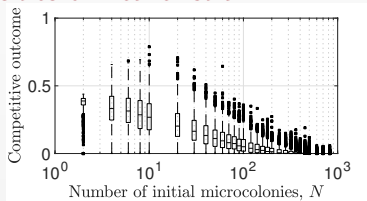
Access to free space predicts outcome

- Access to free space determines competitive outcome in the absence of any other competitive dynamics (isogenic strains).
- Slope of relation between access to free space and competitive outcome depends on founder density.
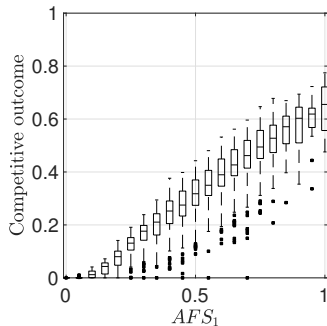

high founder density

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.

low founder density high founder density

Experiment Math. model Experiment Math. model

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 kin discrimination occurs ⇒ "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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