Mathematical Modelling of Pattern Formation in Yeast Biofilms

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About Me

- 2011–2015: UG/Honours, University of Adelaide
  - Free surface flow over topography
- 2016–2019: PhD, University of Adelaide
  - **Modelling yeast biofilm growth**

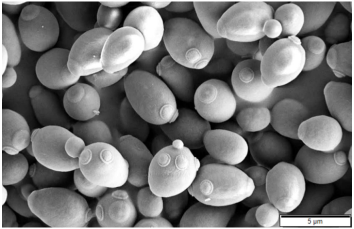
- Sep 2019–present: Postdoc, UQ
  - Modelling actomyosin networks in the cell cortex

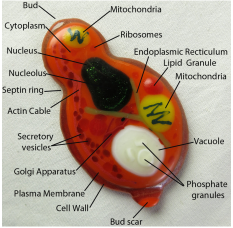
 

- Twitter: @xelamaths

Yeast

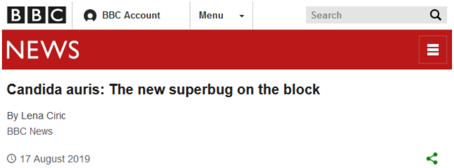
- Single-cell fungi used in food and drink production (beer, wine, bread, vegemite)
- Bakers' yeast is a common model organism
  - Shares important characteristics with plant and animal cells
  - First eukaryotic genome to be completely sequenced
  - Helps develop antifungals and understand (cancer) cell division





Fungal Infections

- Pathogenic yeasts (*e.g. Candida albicans*) colonise medical devices and cause persistent infections
  - Resist antimicrobial therapy — expensive surgery often needed
  - Especially dangerous to immunocompromised people
  - Affects 1–2% of ICU patients, with up to 40% mortality rate[1]
- Emerging pathogen *C. auris*: Japan 2009, 5 continents since
  - Highly resistant and difficult to diagnose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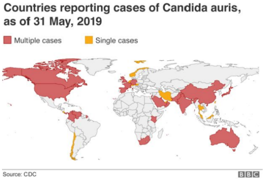
Candida auris: The new superbug on the block
By Lena Ciric
BBC News
17 August 2019

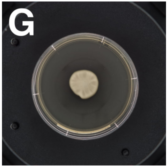


- We seek common mechanisms underlying yeast biofilm growth

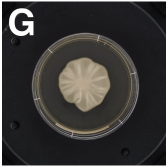
[1] P. G. Pappas et al., *Nat. Rev. Dis. Primers* 4 (2018), 18026.

Yeast Biofilms

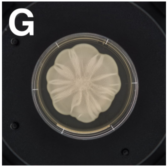
- To help them survive, pathogenic yeasts form **biofilms**: sticky communities of cells and fluid existing on surfaces
- Lab-grown biofilms of bakers' yeast form a floral pattern[2]



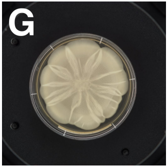
(a) Day 3



(b) Day 5



(c) Day 7



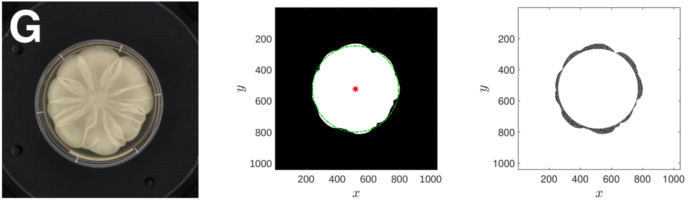
(d) Day 10

- Mechanisms of floral pattern formation only understood qualitatively
  - Nutrient-limited growth
  - Mechanical forces (*e.g.* extracellular fluid flow, adhesion, surface tension)

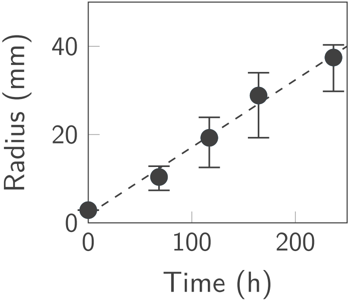
[2] T. B. Reynolds and G. R. Fink, *Science* 291 (2001), pp. 878–881.

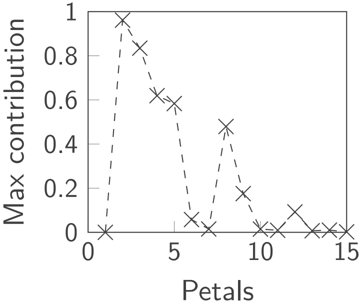
Quantifying Biofilm Patterns

- We ran 13 experiments, and took 4 photographs of each



- Use spatial statistics to quantify biofilm size and shape





Modelling Pattern Formation

Biofilm

Reaction–diffusion

- Alan Turing, 1952
- Movement & consumption of nutrients, cell proliferation
- Well-established analytical methods

Mechanochemical

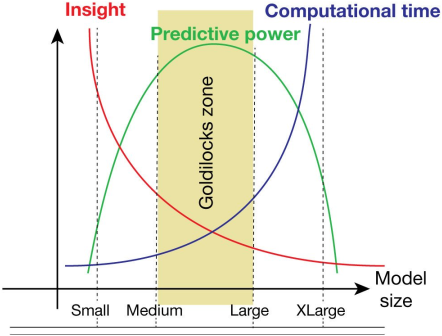
- James Murray, 1980s
- Mechanical/chemical interactions between cells and environment (*e.g.* adhesion, fluid mechanics)
- More detail, complexity

Modelling Pattern Formation

Biofilm

Reaction–diffusion

Mechanochemical



Nutrient-Limited Growth: Reaction–Diffusion Model

- Reaction–diffusion system with non-linear degenerate diffusion for cell spread
  - Enables cell density profiles with compact support
  - Models random motion of cells with non-unity aspect ratio[3]
  - $n(\boldsymbol{x}, t)$: numerical cell density
  - $g(\boldsymbol{x}, t)$: nutrient concentration
  - D: diffusion coefficient ratio, D_n/D_g
- Consider planar geometry accurate for $r \to \infty$

$$\frac{\partial n}{\partial t} = D \frac{\partial}{\partial x}\left(n \frac{\partial n}{\partial x}\right) + ng$$

$$\frac{\partial g}{\partial t} = \frac{\partial^2 g}{\partial x^2} - ng$$

[3] M. J. Simpson, R. E. Baker, and S. W. McCue, *Phys. Rev. E* 83 (2011), 0121901.

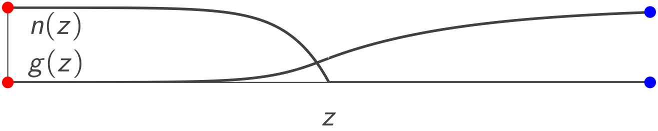
Travelling Wave Analysis

- Travelling waves are a possible explanation for constant-speed expansion
- Introducing the travelling wave co-ordinates $z = x - ct$ and applying BCs yields a system of ODEs
- Defining $\zeta = \int_0^z n^{-1}\,\mathrm{d}s$ removes singularity as $n \to 0$

$n = 1$, $g = 0$, $w = 0$

$$n\frac{\mathrm{d}n}{\mathrm{d}z} = \frac{1}{D}\left(c - cn - w - cg\right)$$
$$\frac{\mathrm{d}g}{\mathrm{d}z} = w$$
$$\frac{\mathrm{d}w}{\mathrm{d}z} = ng - cw$$

$n = 0$, $g = 1$, $w = 0$



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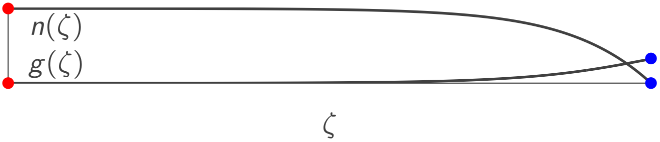
$n = 1$, $g = 0$, $w = 0$

$$\frac{\mathrm{d}n}{\mathrm{d}\zeta} = \frac{1}{D}\left(c - cn - w - cg\right)$$

$$\frac{\mathrm{d}g}{\mathrm{d}\zeta} = nw$$

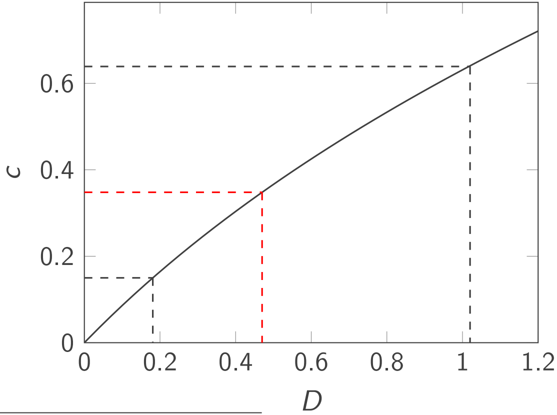
$$\frac{\mathrm{d}w}{\mathrm{d}\zeta} = n^2 g - cnw$$

$n = 0$, $g = g_0$, $w = 0$



Estimating the Diffusion Ratio

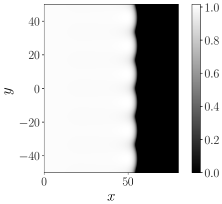
- There is a unique[4] (minimum) wave speed c corresponding to each D
- We estimate D using experimental expansion speed
- Mean data: $D = 0.47$; Experimental range: $D \in [0.18, 1.02]$

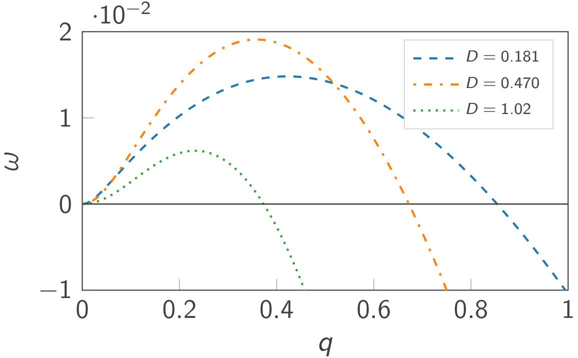


[4] J. Müller and W. van Saarloos, *Phys. Rev. E* 65 (2002), 061111.

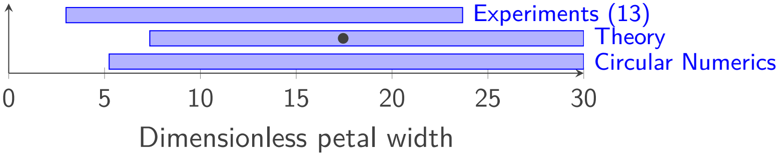
2D Linear Stability Analysis

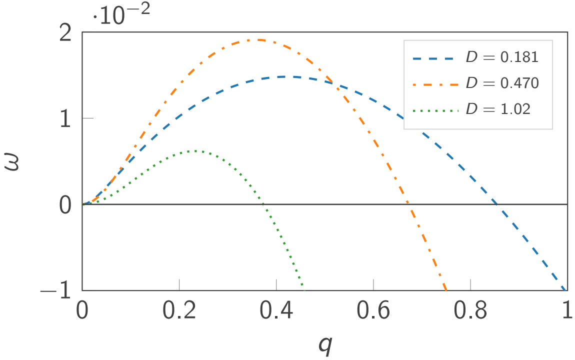
$$\xi = z + \varepsilon e^{iqy+\omega t}$$
$$n(\xi, y, t) = n_0(\xi) + \varepsilon n_1(\xi) e^{iqy+\omega t} + \mathcal{O}(\varepsilon^2)$$
$$g(\xi, y, t) = g_0(\xi) + \varepsilon g_1(\xi) e^{iqy+\omega t} + \mathcal{O}(\varepsilon^2)$$





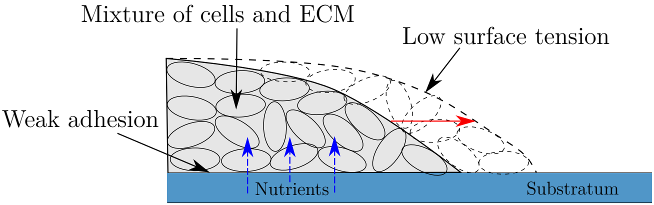
2D Linear Stability Analysis





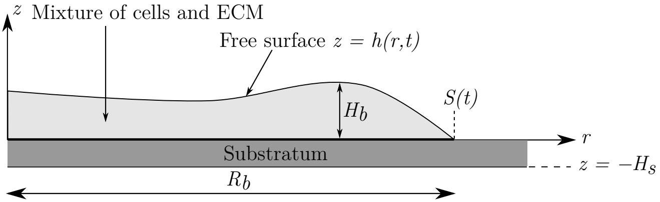
Sliding Motility

- We now consider mechanics in addition to nutrient limitation
- One hypothesis is that yeast biofilms expand by sliding motility[5]
  - Yeast adheres weakly to substratum — enables radial growth as cells proliferate
  - Biofilm takes up nutrients from the substratum
  - Nutrient consumption produces new cells and extracellular fluid
  - Cells and fluid spread passively as a unit



[5] T. B. Reynolds and G. R. Fink, *Science* 291 (2001), pp. 878–881.

Two-Phase Fluid Model



- Axisymmetric cylindrical geometry.
  - Biofilm occupies $0 \le r \le S(t)$ and $0 \le z \le h(r,t)$
- Biofilm is a mixture of two Newtonian viscous fluid phases:
  - Living cells $\phi_n(r,z,t)$ and ECM $\phi_m(r,z,t)$, with $\phi_n + \phi_m = 1$
  - Similar physical properties: $\rho_n = \rho_m$, $\mu_n = \mu_m$, *etc.*
  - Large interphase drag: $\boldsymbol{u}_n = \boldsymbol{u}_m$
- No tangential stress on biofilm–substratum interface
- Thin aspect ratio

$$\frac{H_s}{R_b} = \varepsilon \ll 1, \qquad \frac{H_b}{R_b} = \mathcal{O}(\varepsilon)$$

Governing Equations

- Mass balance (fluid phases)

$$\frac{\partial \phi_n}{\partial t} + \boldsymbol{\nabla} \cdot (\phi_n \boldsymbol{u}) = \psi_n \phi_n g_b - \psi_d \phi_n$$

$$\frac{\partial \phi_m}{\partial t} + \boldsymbol{\nabla} \cdot (\phi_m \boldsymbol{u}) = \psi_m \phi_n g_b + \psi_d \phi_n$$

- Mass balance (nutrients in the substratum and biofilm)

$$\frac{\partial g_s}{\partial t} = D_s \boldsymbol{\nabla}^2 g_s$$

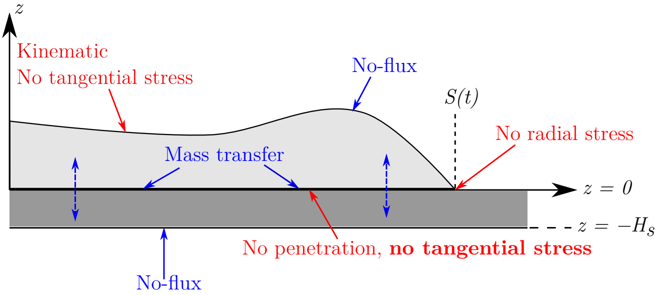
$$\frac{\partial g_b}{\partial t} + \boldsymbol{\nabla} \cdot (g_b \phi_m \boldsymbol{u}) = D_b \boldsymbol{\nabla}^2 g_b - \eta \phi_n g_b$$

- Momentum balance (fluid mixture)

$$\boldsymbol{\nabla} \cdot \boldsymbol{\sigma} = \boldsymbol{0}$$

Boundary Conditions

- Boundary conditions for nutrients and fluids close the model



- Nutrient transfer conditions on $z = 0$:

$$D_s \frac{\partial g_s}{\partial z} = -Q\left(g_s - g_b\right), \quad D_b \frac{\partial g_b}{\partial z} = -Q\left(g_s - g_b\right)$$

- No tangential stress on the substratum models weak adhesion
- Free surface normal stress proportional to local curvature:

$$\widehat{\boldsymbol{n}} \cdot \left(\phi_\alpha \boldsymbol{\sigma} \cdot \widehat{\boldsymbol{n}}\right) = -\gamma\kappa \quad \text{on} \quad z = h$$

Extensional Flow Scaling

- Scaling based on relevant physics
  - Thin biofilm (aspect ratio $\varepsilon \ll 1$)
  - Low surface tension
  - Nutrient-limited growth
- Variables

$$(r, z) = (R_b \hat{r}, \varepsilon R_b \hat{z}), \quad (u_r, u_z) = (\psi_n G R_b \hat{u}_r, \varepsilon \psi_n G R_b \hat{u}_z),$$

$$t = \frac{\hat{t}}{\psi_n G}, \quad g_s = G \hat{g}_s, \quad g_b = G \hat{g}_b, \quad p = \psi_n G \mu \hat{p}$$

- Parameters (estimated based on experiments)

$$\Psi_m = \frac{\psi_m}{\psi_n} = 0.11, \quad \Psi_d = \frac{\psi_d G}{\psi_n} = 0, \quad \gamma^* = \frac{\varepsilon \gamma}{\Psi_n G R_b \mu} = 0,$$

$$D = \frac{D_s}{\psi_n G R_b^2} = 4.34, \quad \mathrm{Pe} = \frac{\psi_n G R_b^2}{D_b} = 0.95, \quad \Upsilon = \frac{\eta R_b^2}{D_b} = 3.15,$$

$$Q_s = \frac{Q R_b}{\varepsilon D_s} = 2.09, \quad Q_b = \frac{Q R_b}{\varepsilon D_b} = 8.65$$

Thin-Film Model

- Expand variables

$$h \sim h_0(r,t)+\varepsilon^2 h_1(r,t), \quad \phi_n \sim \phi_{n0}(r,z,t)+\varepsilon^2\phi_{n1}(r,z,t), \qquad etc.$$

- Dimensionless model (dropping hats)

$$\frac{1}{r}\frac{\partial}{\partial r}(ru_r)+\frac{\partial u_z}{\partial z}=(1+\Psi_m)\,\phi_n g_b$$

$$\frac{\partial \phi_n}{\partial t}+\frac{1}{r}\frac{\partial}{\partial r}(ru_r\phi_n)+\frac{\partial}{\partial z}(u_z\phi_n)=\phi_n g_b-\Psi_d\phi_n$$

$$\frac{\partial g_s}{\partial t}=D\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial g_s}{\partial r}\right)+\frac{1}{\varepsilon^2}\frac{\partial^2 g_s}{\partial z^2}\right]$$

$$\mathrm{Pe}\left(\frac{\partial g_b}{\partial t}+\boldsymbol{\nabla}\cdot[(1-\phi_n)g_b\boldsymbol{u}]\right)=\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial g_b}{\partial r}\right)+\frac{1}{\varepsilon^2}\frac{\partial^2 g_b}{\partial z^2}-\Upsilon\phi_n g_b$$

$$-\frac{\partial p}{\partial r}+\frac{2}{r}\frac{\partial}{\partial r}\left(r\frac{\partial u_r}{\partial r}\right)-\frac{2}{3}\frac{\partial}{\partial r}\left[\frac{1}{r}\frac{\partial}{\partial r}(ru_r)+\frac{\partial u_z}{\partial z}\right]+\frac{\partial}{\partial z}\left(\frac{\partial u_z}{\partial r}+\frac{1}{\varepsilon^2}\frac{\partial u_r}{\partial z}\right)-\frac{2}{r^2}u_r=0$$

$$-\frac{\partial p}{\partial z}+2\frac{\partial^2 u_z}{\partial z^2}-\frac{2}{3}\frac{\partial}{\partial z}\left[\frac{1}{r}\frac{\partial}{\partial r}(ru_r)+\frac{\partial u_z}{\partial z}\right]+\frac{1}{r}\frac{\partial}{\partial r}\left[r\left(\frac{\partial u_r}{\partial z}+\varepsilon^2\frac{\partial u_z}{\partial r}\right)\right]=0$$

Thin-Film Model

- Expand variables

$$h \sim h_0(r,t) + \varepsilon^2 h_1(r,t), \quad \phi_n \sim \phi_{n0}(r,z,t) + \varepsilon^2 \phi_{n1}(r,z,t), \qquad etc.$$

- Simplified leading-order model

$$\frac{1}{r}\frac{\partial}{\partial r}(r u_{r0}) + \frac{\partial u_{z0}}{\partial z} = (1+\Psi_m)\,\phi_{n0} g_{b0}$$

$$\frac{\partial \phi_{n0}}{\partial t} + \frac{1}{r}\frac{\partial}{\partial r}(r u_{r0}\phi_{n0}) + \frac{\partial}{\partial z}(u_{z0}\phi_{n0}) = \phi_{n0} g_{b0} - \Psi_d \phi_{n0}$$

$$\frac{\partial^2 g_{s0}}{\partial z^2} = 0$$

$$\frac{\partial^2 g_{b0}}{\partial z^2} = 0$$

$$\frac{\partial^2 u_{r0}}{\partial z^2} = 0$$

$$-\frac{\partial p_0}{\partial z} + \frac{1}{3}\frac{\partial}{\partial z}\left[\frac{1}{r}\frac{\partial}{\partial r}(r u_{r0}) + \frac{\partial u_{z0}}{\partial z}\right] + \frac{\partial^2 u_{z0}}{\partial z^2} = 0$$

Thin-Film Model

- Integrating across biofilm depth eliminates z dependence

$$\bar{\phi}_n = \frac{1}{h}\int_0^h \phi_n \,\mathrm{d}z.$$

- Applying BCs gives a 1D system for $r \in [0, S(t)]$

$$\frac{\partial h_0}{\partial t} + \frac{1}{r}\frac{\partial}{\partial r}\left(r u_{r0} h_0\right) = \left(1+\Psi_m\right)\bar{\phi}_{n0} g_{b0} h_0$$

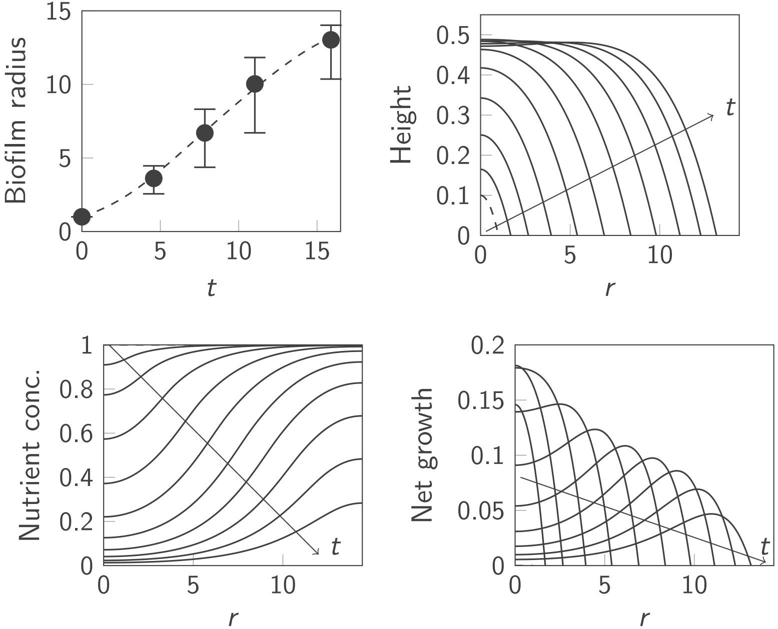
$$\frac{\partial \bar{\phi}_{n0}}{\partial t} + u_{r0}\frac{\partial \bar{\phi}_{n0}}{\partial r} = \bar{\phi}_{n0}\left[g_{b0} - \Psi_d - \left(1+\Psi_m\right)\bar{\phi}_{n0} g_{b0}\right]$$

$$\frac{\partial g_{s0}}{\partial t} = D\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial g_{s0}}{\partial r}\right) - Q_s\left(g_{s0} - g_{b0}\right)\right]$$

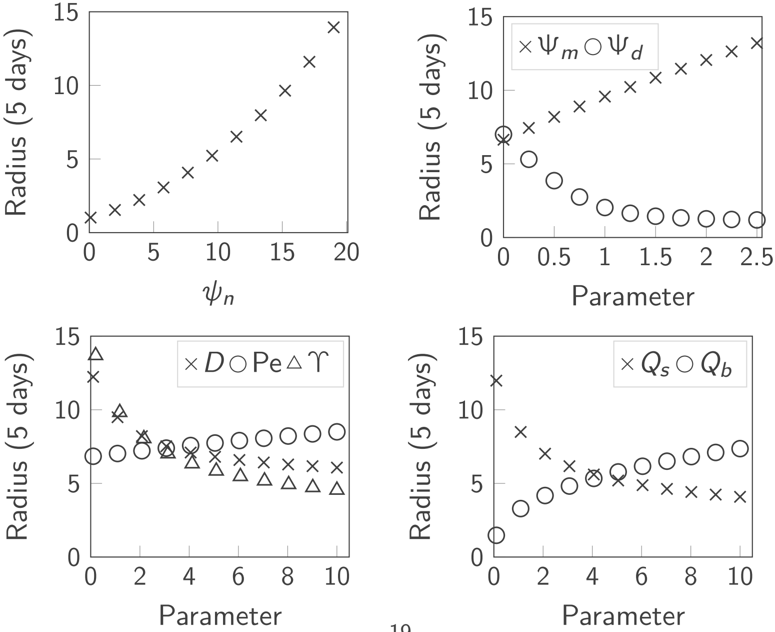
$$\mathrm{Pe}\left[h_0\frac{\partial g_{b0}}{\partial t} + \frac{1}{r}\frac{\partial}{\partial r}\left(r u_{r0}\left(1-\bar{\phi}_{n0}\right) g_{b0} h_0\right)\right] = \frac{1}{r}\frac{\partial}{\partial r}\left(r h_0 \frac{\partial g_{b0}}{\partial r}\right) + Q_b\left(g_{s0} - g_{b0}\right) - \Upsilon \bar{\phi}_{n0} g_{b0} h_0$$

$$4\frac{\partial}{\partial r}\left[\frac{h_0}{r}\frac{\partial}{\partial r}\left(r u_{r0}\right)\right] - 2\frac{u_{r0}}{r}\frac{\partial h_0}{\partial r} = 2\left(1+\Psi_m\right)\frac{\partial}{\partial r}\left(\bar{\phi}_{n0} g_{b0} h_0\right) - \gamma^* h_0 \frac{\partial}{\partial r}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial h_0}{\partial r}\right)\right]$$

Numerical Solutions

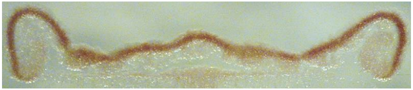


Effect of Parameters on Expansion Speed

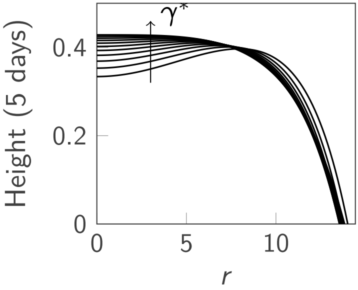


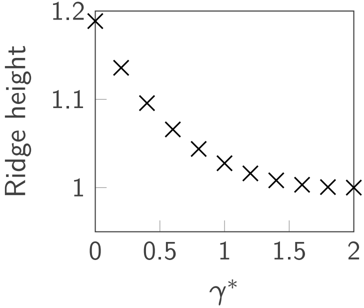
What About Surface Tension?

- In different experiments, yeast colonies can contain ridges[6]



- Surface tension does not affect biofilm size, but can inhibit ridge formation





[6] J. Maršíková et al., *BMC Genom.* 18 (2017), pp. 1–16.

Summary

- Yeast biofilms are a leading cause of bloodstream infections
- We modelled two hypothesised biofilm growth mechanisms
  - Nutrient-limited growth
  - Sliding motility
- Reaction–diffusion model with nonlinear degenerate cell diffusion could explain expansion speed and floral pattern[7]
- Two-phase thin-film fluid model for sliding motility predicts expansion in greater detail[8]
- Future work: 2D solutions to the fluid model

Acknowledgements:

- Supervisors and co-authors
- Funding: A. F. Pillow Trust and Research Training Program

[7] A. Tam et al., *J. Theor. Biol.* 448 (2018), pp. 122–141.
[8] A. Tam et al., *Proc. Royal Soc. A* 475 (2019), 20190175.

UQ Project: Actomyosin Networks

- Actin and myosin interactions in the cortex govern cell shape, movement, and division
- In experiments, disordered actomyosin networks contract
- Mechanisms of contractile stress generation currently disputed