Modeling and Simulation of Bacterial Colony Growth with Cell-Cell Mechanical Interactions

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Founding

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Quick Facts about Bacteria

- Simplest form of life: single-celled, grow, divide, and die.
- 10,000 ~ 100,000 types; rods, spheres, and spirals; 0.5 ~ 5 \( \mu \text{m} \) in size.
- 40 M bacterial cells / gram of soil, and 1 M / ml of fresh water.
- Human body: bacterial cells are 10 times as many as human cells.
- Only 1% of bacteria are found to be harmful.
- Diseases: infections, pneumonia, HIV, etc. Treatment: antibiotics.

Highly Dense Bacteria (Biofilms)

- Push around instead of swimming or swarming.
- Compete for resources and space.
- Communicate with quorum sensing.
- Produce extra-cellular, viscoelastic, multi-component, polymer matrix to stick together.

Staphylococcus aureus: wounds, respiratory tract, food poisoning.
We focus on:

**Growth of *E. coli* on hard agar**

- No swimming and no swarming
- Expansion only through force of growth
- Minimal quorum sensing
- No extracellular polymer matrix
- Metabolism in crowded environment

**Goals**

- Explain experimental findings in terms of growth scaling laws.
- Identify key parameters that control the growth and morphologies.
- Understand the genetic origins of bacterial growth and patterns.
## Different Models

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<th>Spreading mechanism</th>
<th>Dimensionality</th>
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<td>Reaction-diffusion</td>
<td>Swarming pattern formation (Ben-Jacob, Levine, Hallatschek, Suel)</td>
<td>Cells swim and diffuse</td>
<td>2D, single limiting nutrient 2D growth</td>
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<td>(Fisher-Kolmogorov)</td>
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<td>Continuum mechanics</td>
<td>Tissue-growth, thin colonies (Julicher, Shraiman, Brenner)</td>
<td>Fluid and (visco-)elastic mechanics</td>
<td>2D, single limiting nutrient 3D growth</td>
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<td>Cellular automata</td>
<td>Biofilms (Wimpenny, Foster)</td>
<td>Non-Newtonian mechanics (rule based expansion)</td>
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<td>Discrete</td>
<td>Microfluidics (Hasty, Levchenko) Colony growth (Hallatschek)</td>
<td>Granular-level forces</td>
<td>2D or 3D, single nutrient 2D, quasi-3D growth</td>
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<td>PDE for growing surface</td>
<td>Colony growth on solid surface (Grimson)</td>
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Outline

- Experiment
- A Two-Scale Computational Model
- Theory of Mechanical Interactions
- Results and Analysis
- Conclusions
Linear radial growth (Pirt 1967; Cooper et al. 1968; Palumbo et al. 1971; Wimpenny 1979)

Fig. 2. Rates of increase in the diameters of *Escherichia coli* colonies on nutrient agar: (a) glucose, 1.28 g./l.; (b) glucose, 5-12 g./l. Medium DMA; temperature 37°.
Growth kinetics
(Pirt 1967)

Fig. 1. Vertical cross-sections through model colonies (not to scale): (a) during initial exponential growth; (b) during phase of constant radial growth rate. The area $\Delta a$ of width $w$ represents the postulated zone of growth.

A view prevalent for half a century.
Colony shape and linear growth in height

(Wimpenny 1981)

Aerobic (circle) and anaerobic (dots) growth.
Experiment (Hwa lab)

Linear growth both radially and vertically

Linear growth laws
A Two-Scale Computational Model

Model assumptions

- Only one type of cells
- Only one type of nutrient
- No waste
- No dead cells
- No extracellular polymer matrix

- Finite-difference grid
- Initialization
  - Agar surface
  - Random cells
  - Nutrient concentration
- Time iteration
Main Loop

Step 1. Generate the cell density and colony boundary.
Step 2. Update the nutrient concentration and the cell growth rate.
Step 3. Simulate the cell growth, division, and movement.

Step 1. Generate the cell density and colony boundary

Volume fraction \( \phi = \frac{\text{sum of volumes of those cells that are inside the grid box}}{\text{volume of grid box}} \)

Cell density \( \rho(x, t) = \phi(x, t) \rho_{\text{cell}} \)

Colony boundary: thresholding the volume fraction.
Step 2. Update the nutrient and growth rate

Update the nutrient

\[
\frac{\partial C_1}{\partial t} = D_1 \Delta C_1 - \frac{\lambda_{\text{max}} P}{Y} \frac{C_1}{C_1 + K_S} \quad \text{in } \Omega_1(t)
\]

\[
\frac{\partial C_2}{\partial t} = D_2 \Delta C_2 \quad \text{in } \Omega_2
\]

Update the local growth rate

\[
\lambda(x, t) = \lambda_{\text{max}} \frac{C_1(x, t)}{C_1(x, t) + K_S}
\]

Numerical methods

- Forward Euler method
- Finite difference for Laplacian with ghost points near boundary
- Iteration by sweeping over grids
- Multi-resolution meshes
Step 3. Simulate the Cell Growth, Division, and Movement

Cell growth

\[
\frac{dl(t)}{dt} = \lambda(c, t)l(t)
\]

\[
\lambda(x, t) = \lambda_{\text{max}} \frac{C_1(x, t)}{C_1(x, t) + K_S}
\]

Cell division

If \( l \geq l_{\text{div}} \) then the cell splits into two cells.

\[
\begin{align*}
l_1 &= \frac{1}{2} l - r + l_{\text{ran}} \eta \\
l_2 &= \frac{1}{2} l - r - l_{\text{ran}} \eta
\end{align*}
\]

(Negligible loss of mass)

Angular velocities:

\[
\omega_1 = (0, 0, 0), \quad \omega_2 = \omega_{\text{ran}}(0, 0, \xi)
\]

Same velocity

Cell movement: Newton’s law of motion velocity-Verlet algorithm

(Forces will be discussed later.)
Theory of Mechanical Interactions
(Granular solids, Tsimring et al. PNAS 2008 – 2D)

Model of *E. coli* cell: sfero-cylinder

Total force

\[
F = F_{cc} + F_{ca} + F_{surf} + F_{visc}
\]

- **cell-cell interaction**
- **cell-agar interaction**
- **viscous force**
- **surface tension**

\[
V_{cell} = \pi r^2 l + \frac{4}{3} \pi r^3
\]

\[
M_{cell} = \rho_{cell} V_{cell}
\]
Cell-cell interaction

\[ F_{cc} = F_{cc,\text{elas}} + F_{cc,\text{diss},n} + F_{cc,\text{diss},t} \]
\[ T_{cc} = (r_{cc} - c) \times F_{cc} \]

\[ F_{cc,\text{elas}} = k_{cc} \sqrt{r \delta_{cc}^{3/2}} n_d \]
\[ F_{cc,\text{diss},n} = -\gamma_{cc,n} M_{\text{eff}} \delta_{cc} (v_{\text{diff}} \cdot n_d) n_d \]
\[ F_{cc,\text{diss},t} = -\min \left\{ \gamma_{cc,t} M_{\text{eff}} \delta_{cc}^{1/2}, \frac{\mu_{cc} k_{cc} \sqrt{r \delta_{cc}^{3/2}}}{|v_{\text{diff}} - (v_{\text{diff}} \cdot n_d) n_d|} \right\} (v_{\text{diff}} - (v_{\text{diff}} \cdot n_d) n_d) \]

\[ \delta_{cc} = 2r - d \]
\[ v_{\text{diff}} = V - V' \]
\[ M_{\text{eff}} = \rho_{\text{cell}} \frac{2V_{\text{cell}} V'_{\text{cell}}}{V_{\text{cell}} + V'_{\text{cell}}} \]

(Hertzian contact force)
Cell-agar interaction

\[ F_{ca} = F_{ca, elas} + F_{ca, diss,n} + F_{ca, diss,t} \]
\[ T_{ca} = (r_{ca} - c) \times F_{ca} \]

\[ F_{ca, elas} = k_{ca} \sqrt{r} \delta_{ca}^{1/2} n_z \]
\[ F_{ca, diss,n} = -\gamma_{ca,n} M \delta_{ca} (v_{ca} \cdot n_z) n_z \]
\[ F_{ca, diss,t} = -\min \left\{ \gamma_{ca,t} M \delta_{ca}^{1/2} \frac{\mu_{ca} k_{ca} \sqrt{r} \delta_{ca}^{3/2}}{|v_{ca} - (v_{ca} \cdot n_z) n_z|} \right\} (v_{ca} - (v_{ca} \cdot n_z) n_z) \]
\[ v_{ca} = v + \omega \times (r_{ca} - c) \]

Viscous force and torque

\[ F_{visc} = -6\pi \mu_{liq} r v \]
\[ T_{visc} = -8\pi \mu_{liq} r^3 \omega \]
Cells are coated with water, in chemical equilibrium with the colony.

A cell sticking out of the colony increases the water-air interfacial area.

Unbalance of surface tension force and cell-agar friction force initiates the buckling.

\[
F_{\text{surf}} = F_{\text{surf},p} + F_{\text{surf},q} \\
T_{\text{surf}} = (p - c) \times F_{\text{surf},p} + (q - c) \times F_{\text{surf},q}
\]

Linear interpolation

\[
F = \frac{dW}{dh} = \frac{\gamma_{\text{surf}} dA}{dh} = 2\pi \gamma_{\text{surf}} r
\]

\[
F_{\text{surf}} = 2\pi \gamma_{\text{surf}} r \min \left\{ \max\left\{0, \delta z \mathbf{n}_z \cdot \mathbf{n}_s + \delta h \right\}, 1 \right\} \mathbf{n}_s
\]
Results and Analysis
Vertical and Radial Growth: Scaling and Colony Shape

- Why linear growth?
- What are the controlling parameters?
Vertical Growth: Orientations

Cyan cells: large angles with the z-axis. Golden cells: smaller angles.

Top view of part of a colony.

A cross section of the colony.

Bottom view of a central part.

Bottom view of a periphery part.
Vertical Growth: Orientation and Growth Zone

(A) angle formed by cells with xy-plane

(B) time since last divided

(C) local growth rate

(D) $h$

(E) time since last divided

(F) local growth rate
A 1D model for the nutrient penetration level and growth zone

\[ D + C'''(z) = \frac{\rho_0 \lambda_S}{Y} \frac{C}{C + K_S} \quad \text{for } z > 0 \]

\[ C(0) = C_0 \quad \text{and} \quad C(\infty) = 0 \]

In a non-dimensionalized form

\[ \tilde{C}(\tilde{z}) \leq e^{-\sqrt{2/3}(\tilde{z} - \tilde{z}_0)} \quad \forall \tilde{z} \geq \tilde{z}_0 \]

\[ \left( \sqrt{\tilde{C}_0} - \frac{1}{\sqrt{2}} \tilde{z} \right)^2 \leq \tilde{C}(\tilde{z}) \leq \left( \sqrt{\tilde{C}_0} - \sqrt{\frac{\ln(e/2)}{2}} \tilde{z} \right)^2 \]

\[ \forall \tilde{z} \in [0, \tilde{z}_0]. \]

Fig. 3D simulations (green *) and 1D prediction (line or circle): semi-log plots.

**Linear vertical growth:** \( V_H \propto H_S \lambda_S. \)

\[ V_{\text{colony}} \propto R^2 H \propto R^3 \quad \Rightarrow \quad V_{\text{growth}} \propto \frac{d}{dt} V_{\text{colony}} \propto R^2 \]

\[ A \text{ disk of thickness } H_S \]

\[ \text{Vertical ascending speed } V_H \propto H_S \lambda_S \]
Radial Growth: Only cells in a ring at the edge grow radially.
Azimuthal averages vs. the distance from the rim

(A) $V_r$ vs. $\Delta r$ for different times ($t=12h$, $t=16h$, $t=20h$) and theoretical line.

(B) $H$ vs. $\Delta r$ for different times ($t=12h$, $t=16h$, $t=20h$).

(C) $V_z$ vs. $\Delta r$ for $t$-averaged values.

(D) $P$ vs. $\Delta r$ for $t=20h$.

(E) $H$ vs. $\Delta r$ for different $\lambda_s$ values ($\lambda_s=1.0h^{-1}$, $\lambda_s=0.6h^{-1}$).

(F) $V_r$ vs. $\lambda_s$ for fixed $I_{\text{div}}$ and variable $I_{\text{div}}$. 

Azimuthal averages vs. the distance from the rim.
Buckling and radial growth rate

Conservation law
\[ \vec{V} \cdot \vec{V} = \lambda \]

Small angular velocity and thin layer
\[ \frac{1}{r} (rV_r)' = \lambda \]

Velocity at the edge
\[ V_r(\Delta r = 0) = V_R \]

\( \Delta r = \) the signed distance from the edge

Define the buckling width \( W_b \) by \( V_r(-W_b) = 0 \)

\[ 0 \approx V_R - \lambda_s \ W_b \]

Linear radial growth: \( V_R \propto \lambda_s \ W_b \)
Conclusions

**Achievement**

- Experiment, theory, and a new, two-scale computational model.
- Mechanisms of linear growth:
  - Constant vertical expansion due to limited nutrient penetration;
  - Constant radial expansion due to growing buckling region.
- Parameters: nutrient; maximum growth rate, frictions, surface tension.

**Summary of Analysis**
Discussions

- Treatment of surface tension.
- Cellular details and continuum description of buckling.
- Time stepping for cell movement.
- Computational limitations: less than 10 million cells.
- Parameter optimization: too many parameters!

Current and Future Work

- Extension: multiple species of cells; different types of nutrients; oxygen; wastes; etc.
- Numerical analysis and fast computation.
- Analysis of buckling.
- Coarse-grained models. Hydrodynamic limit.
- Complex patterns: smoking, turbulence, etc.
THANK YOU!