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Bacterial Colony growth - Theoretical overview and computational models. [1]

Biophysics I WS23/24

Hari prasad Sreekrishnapurath Variyam[†]

[†]Graduate Student,
Department of Physics
University of Cologne

hsreekri@smail.uni-koeln.de

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Motivation I

Why study bacterial growth?

- Bacterial infection and spread through living tissues
- Antibiotic resistance of colonies.
- Understanding other bio-geochemical cycles that involves bacterial growth
- Effective waste water treatment.
- Petroleum processing

How mathematical/statistical models can help

- Statisical physics in general deals with ensemble systems which has complex behaviours moderated by microscopic fluctutations.
- Turns out bacterial growth is an excellent model problem, statistical toy models can be implemented effectively.

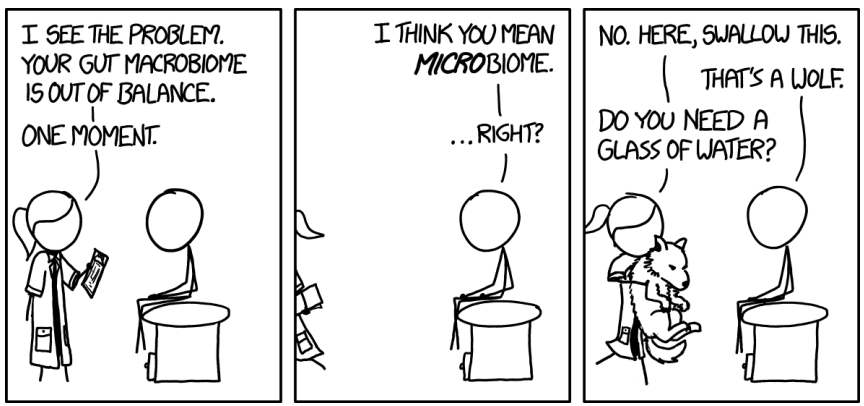


Figure: *Credits : xkcd.com*

Motivation II

Bacterial growth in a statistical lens

- From a statistical viewpoint, a bacterium can be visualized as a microscopic particle or a cell. Specifically each cell constitutes of a cell wall, and a 'soup' of essential biomolecules, which includes DNA, RNA, proteins etc.
- The growth of a bacterial cluster can essentially be thought of as a process of conversion of chemical nutrients into biomass.
- The increase in biomass is accompanied by an increase in cell size and replication of the bacterial DNA (which can contain possible errors (mutations)) which eventually leads to the splitting of the cell into equal divisions which is often termed as binary fission.

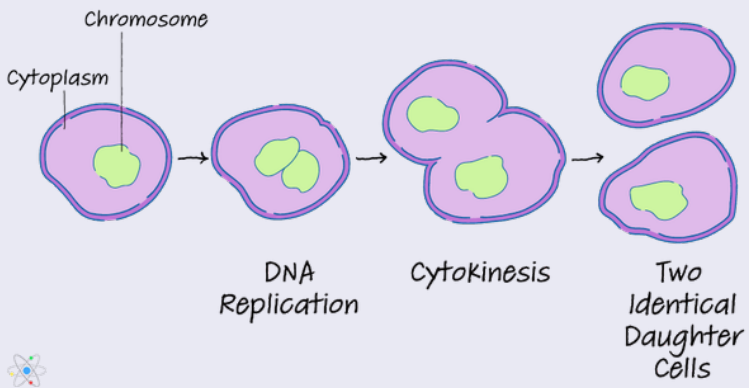


Figure: Binary fission - *credits : - Sciencing.com*

Examples of bacterial colony evolution

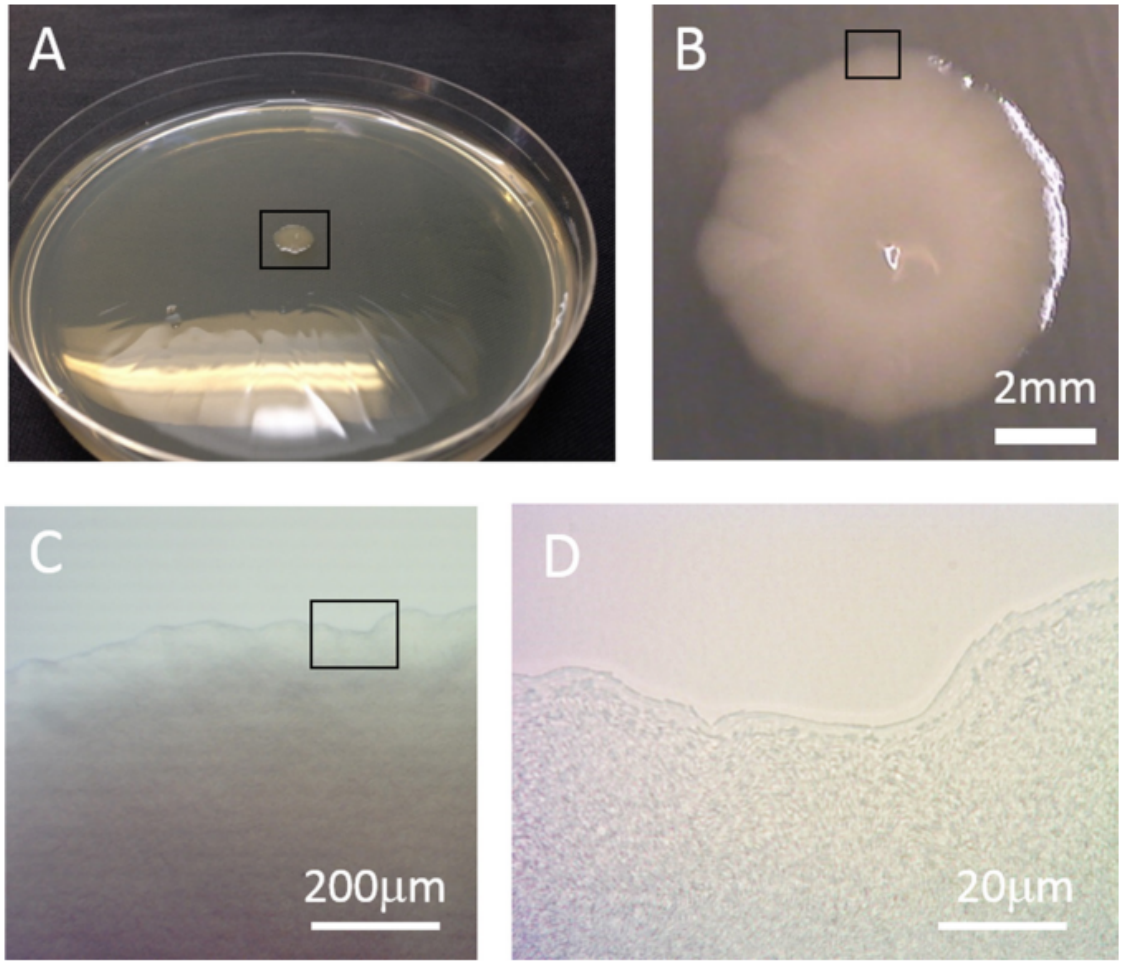


Figure: Bacterial colony evolution of *E. Coli*

Modelling spatially homogeneous colonies I

Unlimited nutrients

- In a spatially homogeneous environment, assuming the nutrient concentration is unlimited, we can write,

$$\frac{dN(t)}{dt} = rN(t) \tag{1}$$

Where $N(t)$ is the number of bacterial colonies at time t and r is the replication rate. Which leads to an exponential solution.

Limited nutrient concentrations

- In case the nutrient concentration is limited, the population goes through a transition phase and saturates, a coupled set of dynamical equations can be written

$$\frac{dN}{dt} = \left(\frac{r_{max}s}{K_s + s}\right) N \tag{2}$$

$$\frac{ds}{dt} = -\gamma \left(\frac{r_{max}s}{K_s + s}\right) N \tag{3}$$

Where K_s is half maximal nutrient concentration, r_{max} is the maximal per cell growth rate, s is the nutrient concentration, and γ is a yield coefficient.

Comparison between exponential and logistic growth

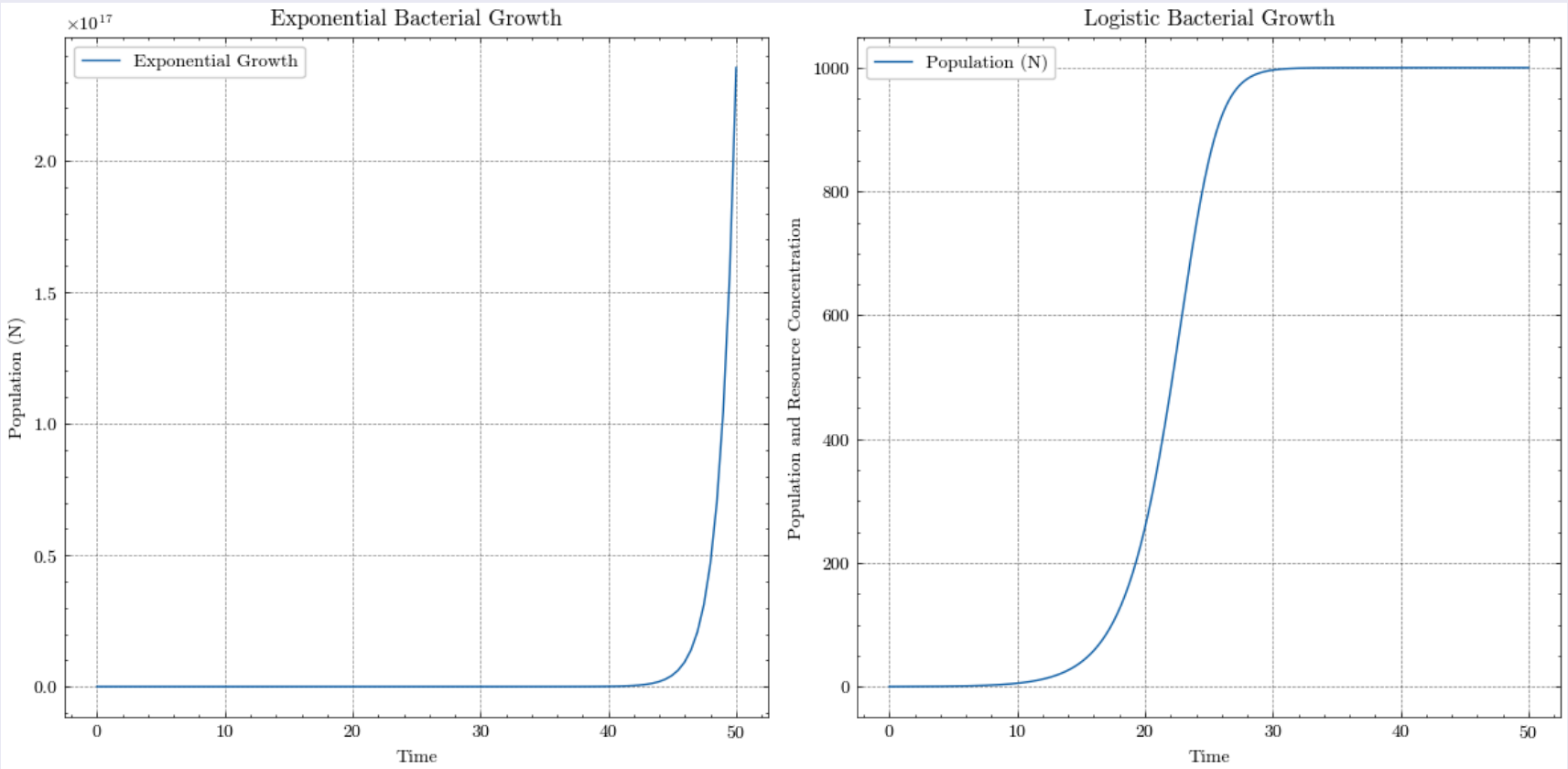


Figure: Exponential vs Logistic growth

Spatio-temporal modelling of colony growth

Individual/Agent based models

- Most of the time, it is not appropriate that the bacterial population is considered as a continuous field, instead a detailed resolution at the level of individual cell level.
- To effectively tackle this, individual models or agent models are developed.
- A simplest form of such a class of models are the lattice based models.
- In this case, a lattice site can be occupied by a bacterial cell, and can evolve with simple set rules.
  - Eden growth models
  - Diffusion limited aggregation (DLA) models.

Continuum models

- Here the bacterial colony is considered to be a continuous field, that is dependant on the spatial heterogeneity.
  - Fisher kolmogorov Petrovsky Piscounov (FKPP) models.

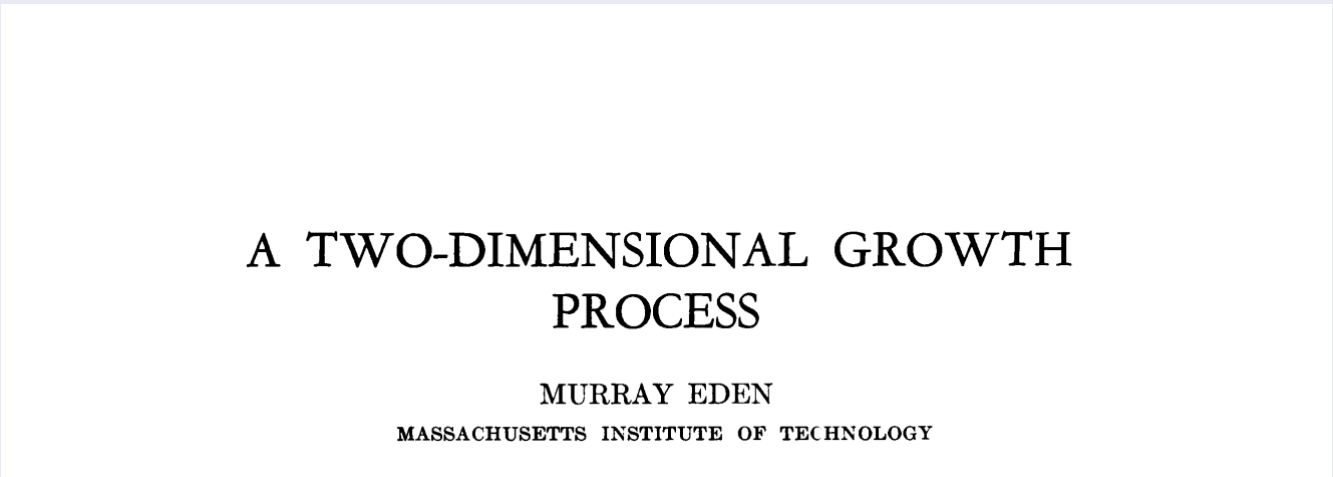
Experimental background

- **Bacterial Strain:** B. subtilis, rod-shaped with flagellae, approximately 0.7 mm in diameter and 2 mm in length.
- **Inoculation:** Bacteria point-inoculated at the center of an agar plate containing peptone as a nutrient in a plastic petri dish (diameter: 88 mm).
- **Agar-Gel Network:** Average pore size of the agar-gel network smaller than the size of bacteria, promoting two-dimensional growth on the agar surface.
- **Wakita et al. (1994):**
  - **Temperature:** Kept constant at 35°C.
  - **Morphology Analysis:** Examined colony morphology as a function of agar concentration (Ca) and nutrient concentration (Cn), while keeping temperature constant.
  - **Agar Concentration (Ca):** Drastic changes observed with variations in Ca. Colony patterns changed from DLA-like (low Cn, region A) to "Eden-like" (high Cn, region B) when Ca was higher than 8 g l1 (hard agar plates).
  - **Nutrient Concentration (Cn):** High Cn resulted in "Eden-like" round and compact colonies with a self-affine fractal growing interface.
  - **DLA-like Branches:** Thickened as Cn increased, eventually fusing together to form a compact pattern.

Eden growth models

Growth rules

- The Eden model is comprised of a simple growth rule, given a seed particle, a particle can randomly stick to any of it's neighbours. Often an 8 cell neighbourhood (Moore neighbourhood) is considered.
- This model was first proposed by Murray Eden, a bio-statistician from MIT



A TWO-DIMENSIONAL GROWTH PROCESS

MURRAY EDEN

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Figure: "A two dimensional growth process" [*Eden 1961*]

Eden growth models II

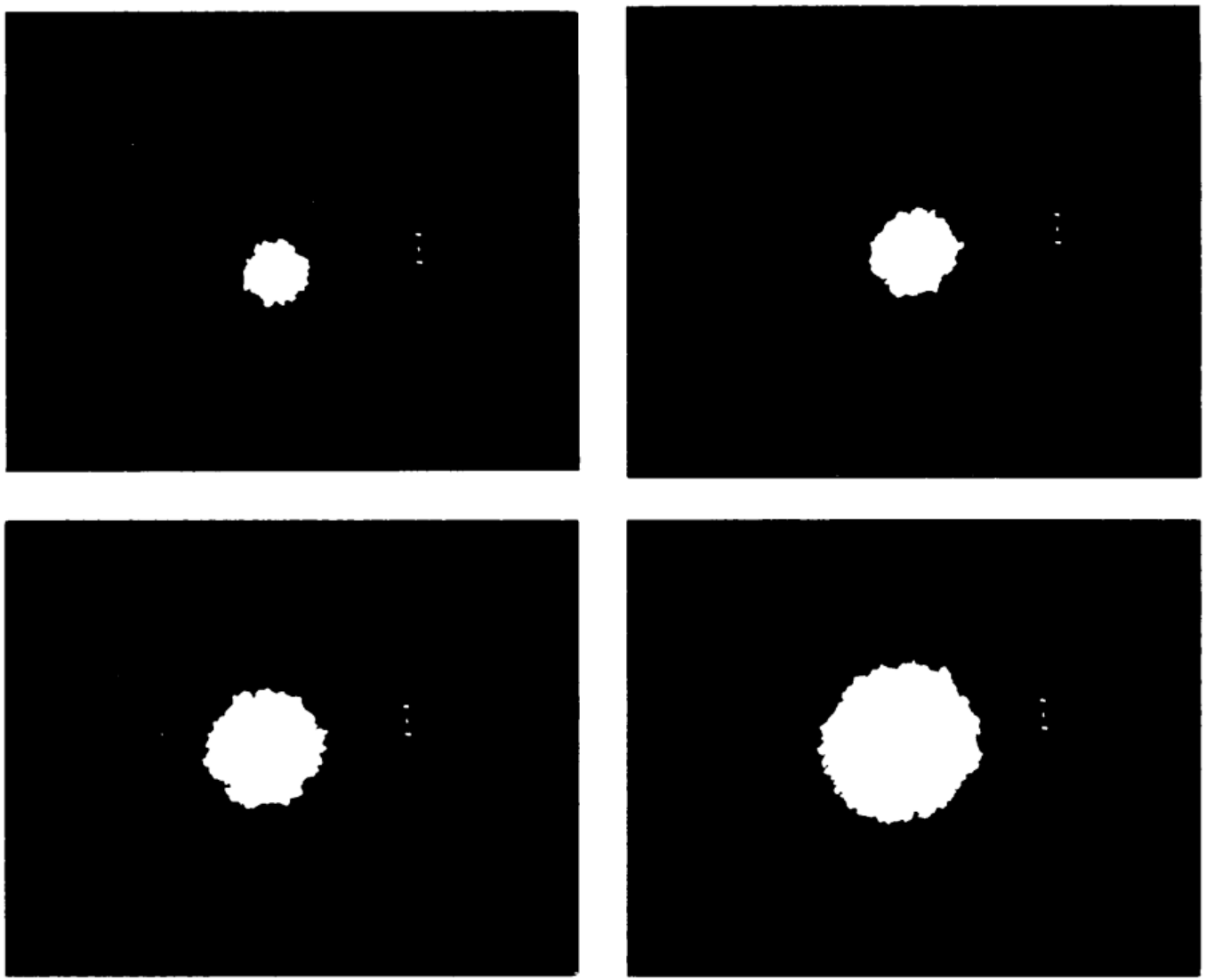


Figure: Different stages of Eden growth [*Eden 1961*]

Simulation

Eden growth models IV

Experimental observations for Eden like models

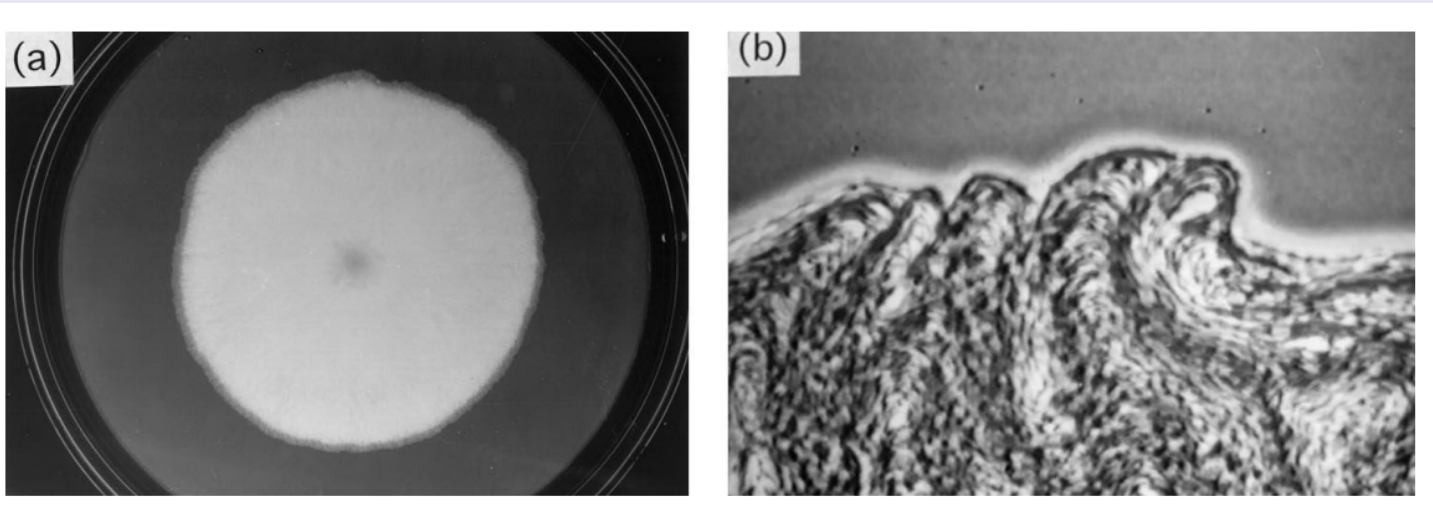


Figure: Experiments done on wild type *B. Subtilis* [*Matsuhita 1990*]

- The roughness exponent α was experimentally obtained to be 0.78 for such colony which matches with the estimation from the model.

Modifications

- Eden growth model can be simulated by different lattices, but lattice geometries can create an anisotropy in the system.
- For more isotropic modelling, off-lattice models has also been explored.

Growth rule

- Compared to Eden growth model, DLA involves slightly more complicated set of rules.
- Initially we start with a seed in the middle, similar to that of the Eden model. Then we start a random walker from the edge of a circle of radius of r that we define.
- This particle excecutes a random walk and if the particle eventually hits the edge, its rejected.
- But if the particle by chance, hits the seed particle, it sticks. This process continues.

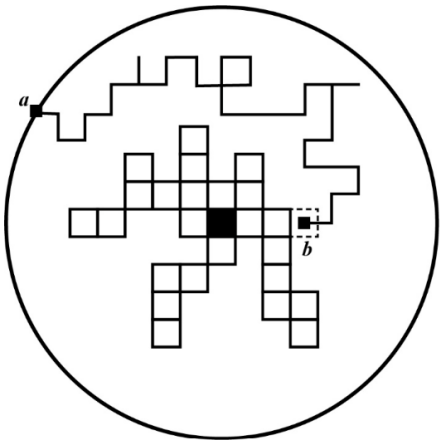


Figure: DLA growth rule

Simulations

DLA III

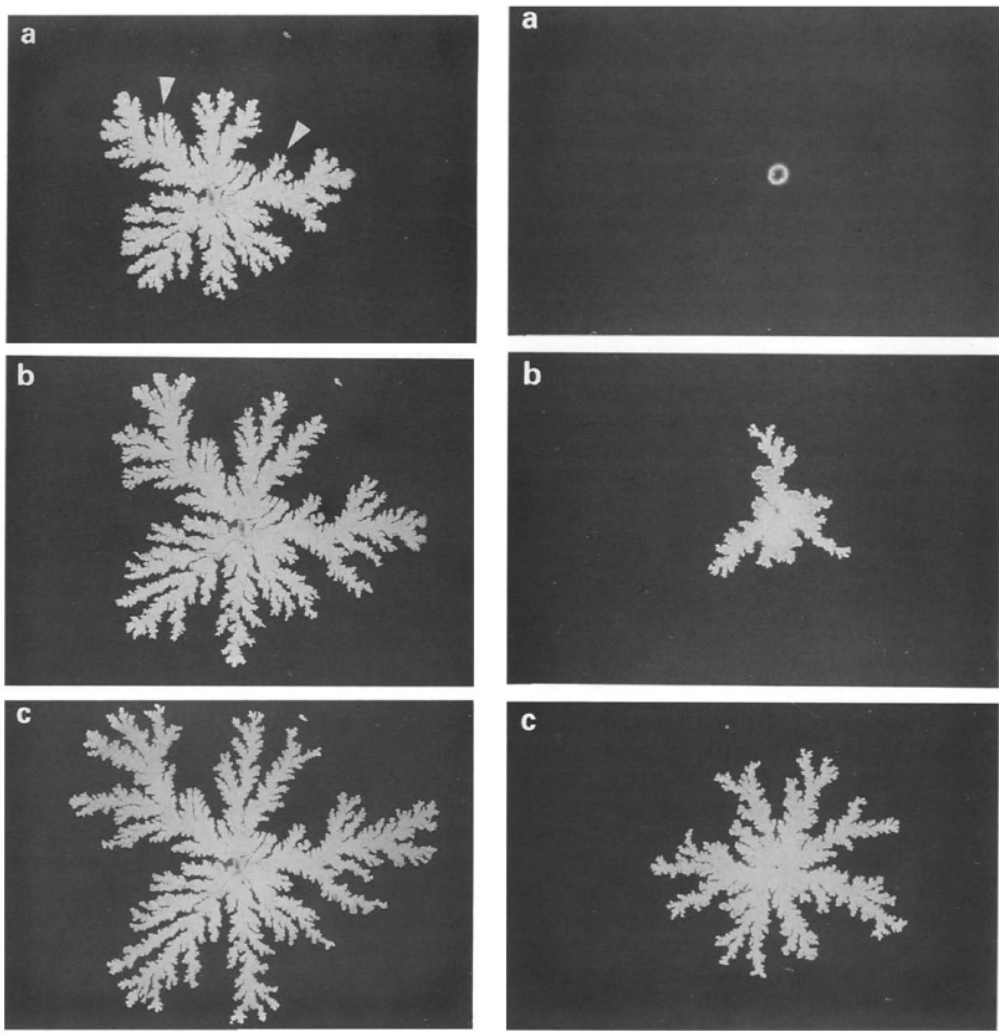


Figure: Experimental observations done on *B. subtilis* by Matsuhita et al. [*Matsuhita 1990*]

Comparisons

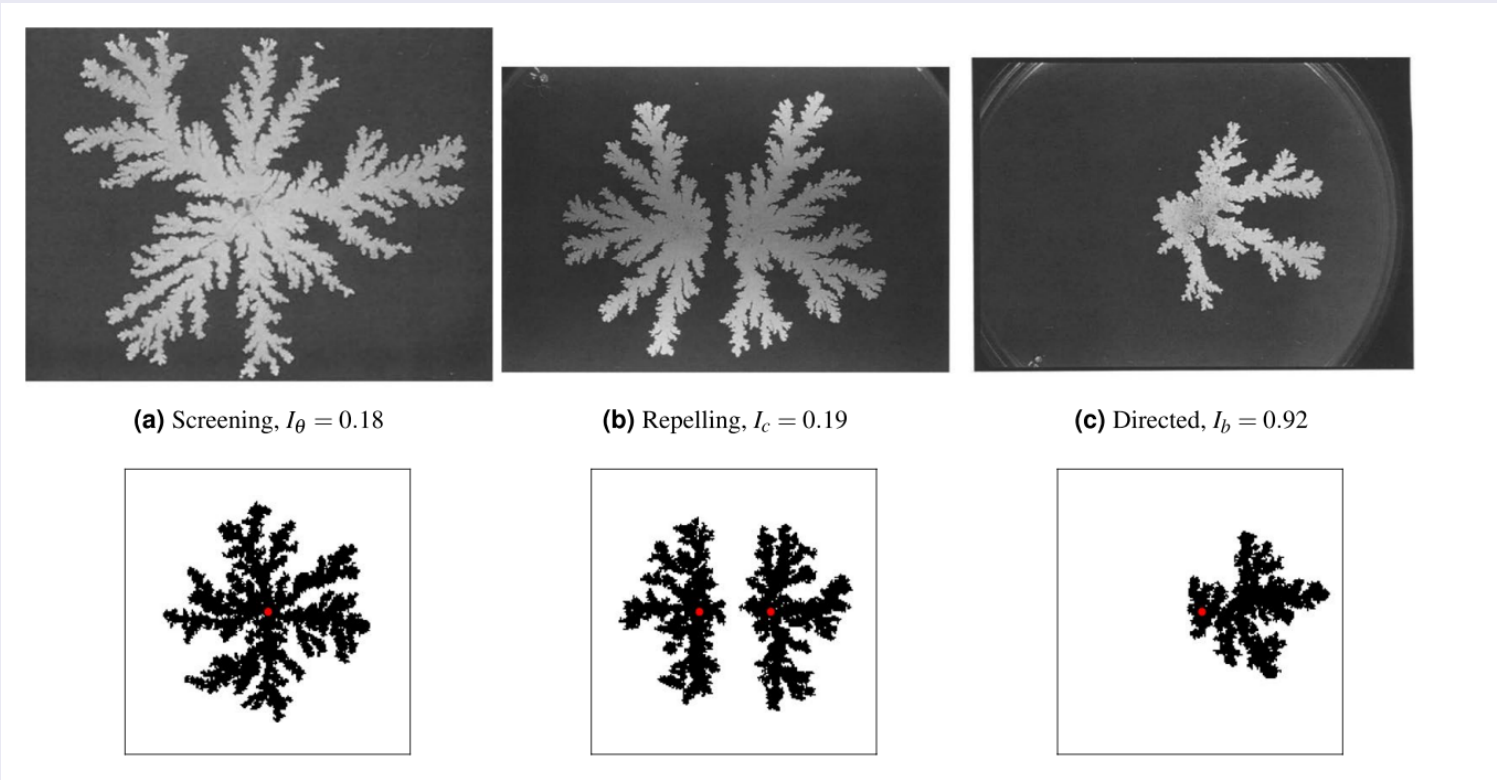


Figure: [*Matsuhita 1990*]

- The experimental and simulated colonies have been plotted side by side apart from visual similarities, the fractal dimension of the colony was calculated and was found to consistent with fractal dimension of the DLA which is 1.72

FKPP model(s)

Mathematical formulation

- The FKPP (Fisher kolmogorov Petrovsky Piscounov) [*Kolmogorov 1937*] model essentially simulates a diffussion equation model, with a logisitic term associated with it which represents a saturation, of the growth after it reaches a cutoff.
- Mathematically we can write this as,

$$\partial_t n = D\nabla^2 n + r(n(1 - \frac{n}{K})) \tag{4}$$

  where D is bacterial colony diffusion coefficient through a medium, and r is the growth rate, and $n(x, t)$ is the colony concentration.
- This also is a homogeneous growth model and we are not considering any stochasticity involved, thus we obtain smooth growth patterns.
- Simulating a 1 dimensional analogue results in the following result.

FKPP II

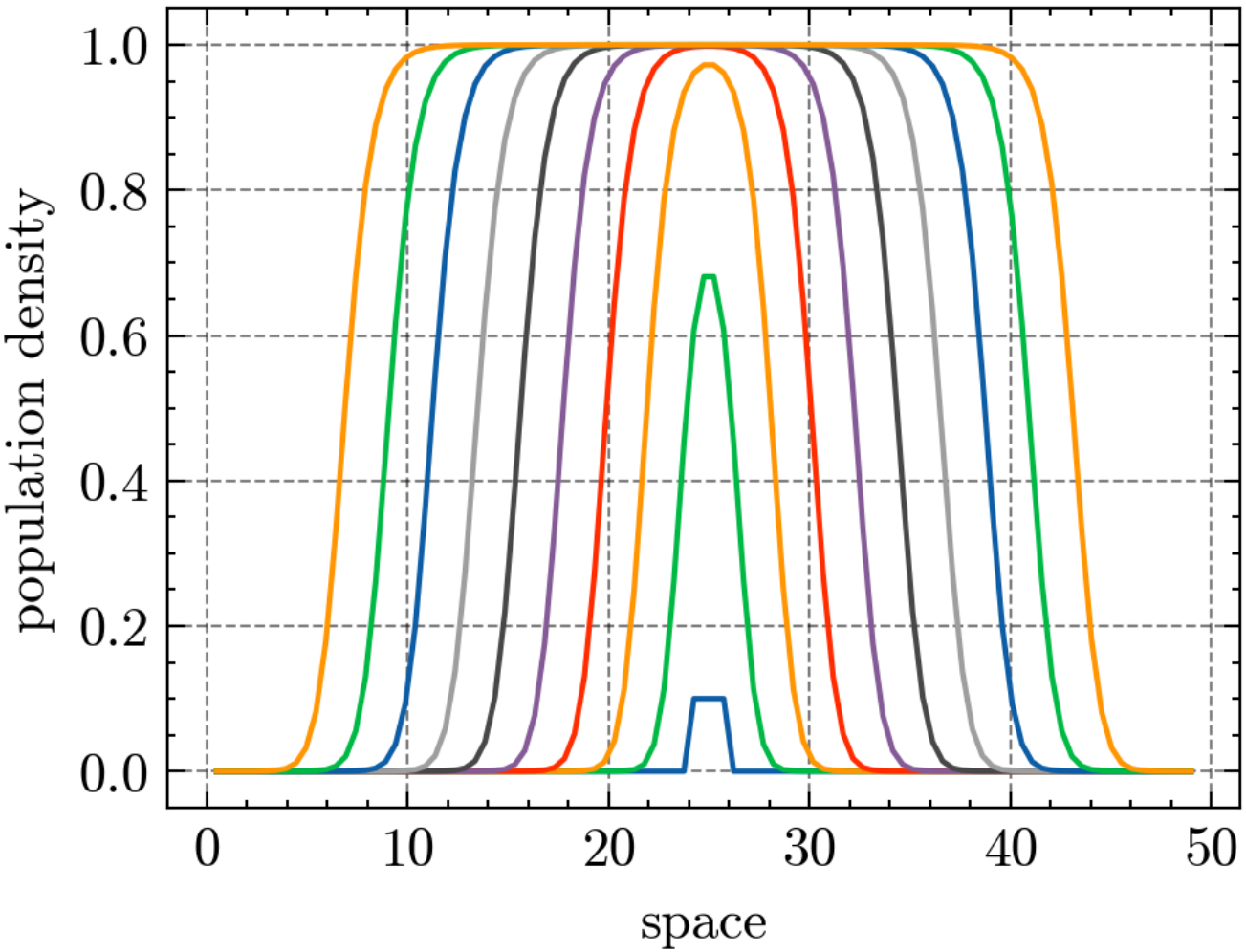


Figure: FKPP in 1D

Further modifications of FKPP

Reaction diffusion models

- FKPP models are in general a reaction diffusion system but with homogeneous conditions, but this doesn't exactly solve the problem of accurately modelling the spatio-temporal morphology bacterial colonies.
- Further generalizations can be added and coupled RD systems can be constructed.

$$\partial_t b = \nabla^2 b + \mathrm{f}(b, n) \tag{5}$$

$$\partial_t n = \nabla^2 n + \mathrm{g}(b, n) \tag{6}$$

Model proposed by Kawasaki et al.

- A reaction diffusion model based on equations (5) and (6) was proposed by Kawasaki et al. which encapsulates many of the bacterial colony growth morphologies.
- Here the RD equations read,

$$\partial_t b = \nabla^2 b - bn \tag{7}$$

$$\partial_t n = \nabla \cdot \{\sigma n b \nabla b\} + nb \tag{8}$$

Simulation parameters and results

- Simulating the partial differential equations, where and initial uniform concentration of nutrients ν_0 and a stochastically varying σ_0 by drawing a value from a triangular distribution $\Delta(-1, 1)$ results various colony morphologies.

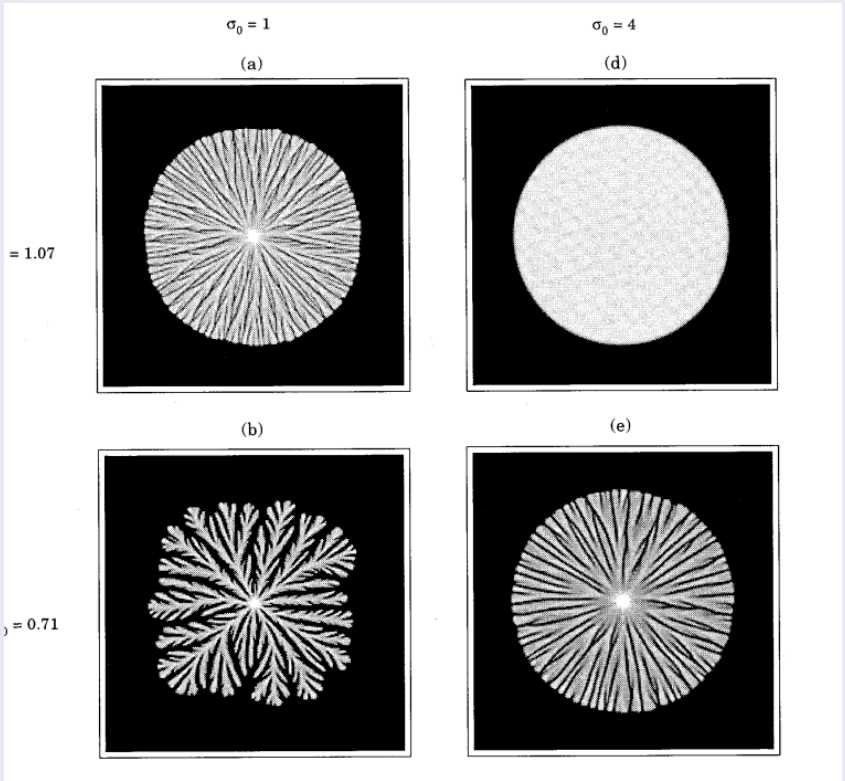


Figure: Different bacterial colony shapes with different initial conditions.
Kawasaki 1997

Conclusions

- So far we have explored different computational models that can model spatio-temporal aspects bacterial colony growth.
- Statistical models while being extremely minimal, offer an excellent level of predictability of colony evolution and thus is significantly useful in case of understanding bacterial growth in different areas mentioned in the introduction.
- All these models only serve as a starting point to create much more robust and tractable models. (We have only scratched the surface!)

Acknowledgements

This presentation was done under the help and guidance of Paul Schiefer.

Thank you

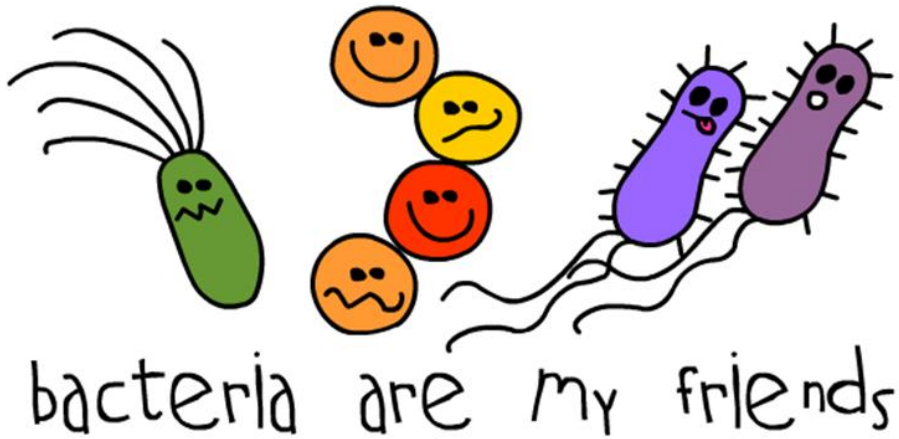


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Figure: Scan me!