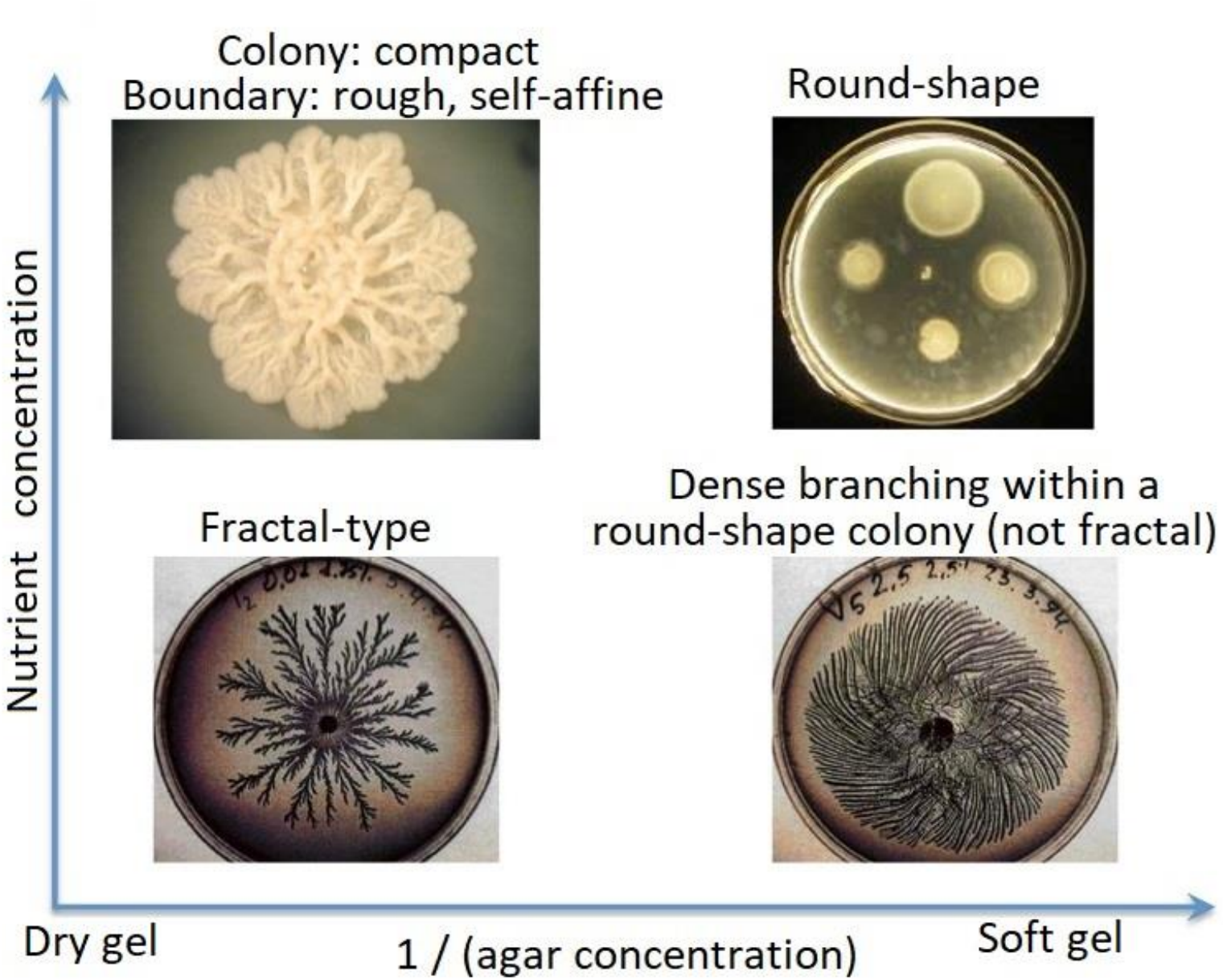
The geometry of bacteria colonies II

Self-affine surfaces, branching morphology and models for individual bacteria

October 9, 2017

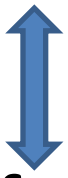
“Summary” of the morphology diagrams



Compact morphology

Abundant nutrient → compact colony

Either smooth or irregular perimeter



Soft gel → - Bacteria can move

- Takes a few hours to migrate across the dish
- Random walk trajectory

⇒ Inter-cellular interactions are negligible

⇒ Time dependence of the bacterial density ρ can be described by the Fisher-Kolmogorov equation

Fisher-Kolmogorov equation

Starts as a small spot, then diffuses due to random translation and multiplication

$$\frac{\partial \rho}{\partial t} = D_\rho \nabla^2 \rho + f(\rho, c)$$

Notations:

$\rho = \rho(\vec{r}, t)$: bacterial density

D_ρ : Diffusion coefficient (can be determined from the (measurable) squared displacements $d^2(t)$ of the individual cells during a time period t as :

$$\overline{d^2(t)} = 2D_\rho t$$

where the overline means averaging among the cells)

∇ : Partial derivative (with respect of the space coordinates)

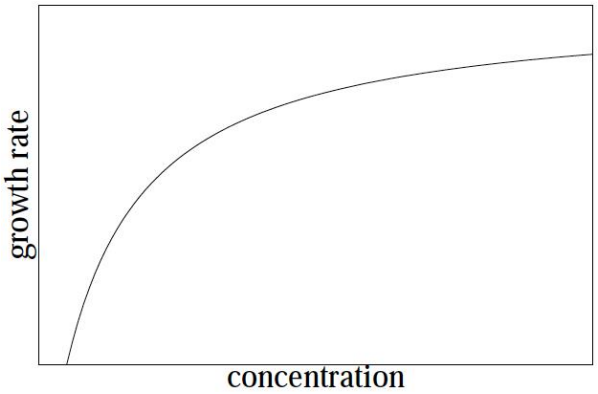
$f = f(\rho, c)$: Bacterial multiplication

c : Nutrient concentration

Fisher-Kolmogorov equation – cont.

Dependency on c :

Hyperbolic manner



R(c)= for small c values $R \sim c$
for big c values R is const.

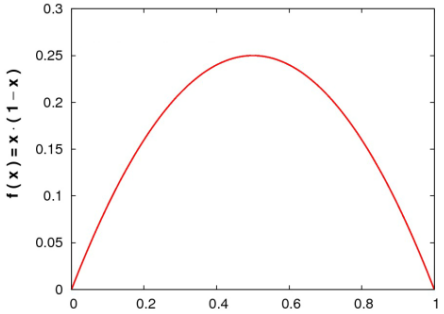
Some amount is needed for maintaining the intracellular biochemical process

Dependency on ρ :

When ρ is small, cells proliferate with a fixed rate

→ exponential growth

In practice, even with unlimited nutrient supply, there's a certain threshold ρ^* for the density (e.g., accumulation of toxic metabolites)



$$f(\rho, c) = R(c) * \rho(1 - \rho)$$

Numerical solution of the Fisher-Kolmogorov equation in 1 D

$$\frac{\partial \rho}{\partial t} = D_\rho \nabla^2 \rho + R(c)\rho(1-\rho)$$

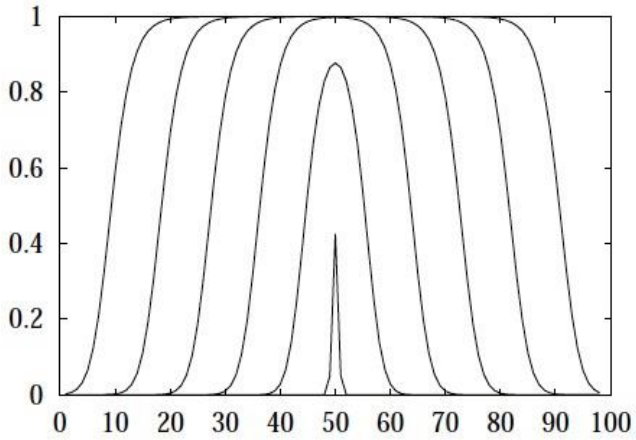


Figure 4.9: Typical result of the numerical integration of the Fisher equation (4.8) starting from a localised perturbation ($D_\varrho = 1$, $f(x) = x - x^2$, i.e., $r = 1$). The $\varrho(x,t)$ curves are plotted for $t = 0, 5, 10, 15, 20$ and 25. The domain grows with a stationary speed of $v = 2$.

Numerical solution: the growing domain of the colony expands with a constant speed $v \approx v_*$ where

$$v_* = 2\sqrt{RD_\rho}$$

Solution of the Fisher-Kolmogorov equation in 2D

The Fisher-KPP equation.mp4

https://www.youtube.com/watch?v=IjKYE5-RhHc

Numerical solution of the Fisher-Kolmogorov equation in 1 D – cont.

- We had: the colony expands with const speed $v \approx v_* = 2\sqrt{RD_\rho}$
- To calculate v, we rewrite the expanding domain of the bacteria density $\rho(x,t)\sim 1$ into a moving frame of reference as $\tilde{\rho}(u,t) = \rho(x,t),$

  where $u = x - vt, v > 0, \rho(-\infty)$=1 and $\rho(\infty)$=0
- Inserting $\tilde{\rho}(u,t)$ into $\frac{\partial \rho}{\partial t} = D_\rho \nabla^2 \rho + R(c)\rho(1-\rho)$, we obtain

$$\frac{\partial \tilde{\rho}}{\partial t} = D_\rho \tilde{\rho}'' + v\tilde{\rho}' + f(\tilde{\rho})$$

(where ' is differentiation with respect to *u*)

- This can be solved analytically: gives stationary solution for any value of $v \geq v_*$.
- "velocity selection problem"
  – not unusual in equations describing pattern formation.

Compact morphology

Abundant nutrient → compact colony

Either smooth or irregular perimeter

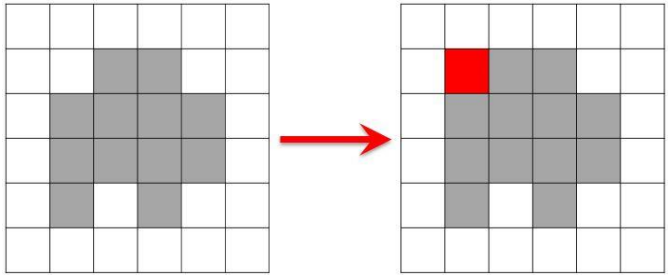
- Dry gel and/or un-motile bacteria
  - Bacteria exert mechanical pressure on their environment (in order to expand to their preferred size)
  - Inter-cellular interactions
  - Modified Fisher-Kolmogorov equation
  - Irregular (self-affine) surface

The formation of self-affine boundaries – the Eden model

- One of the earliest method to generate self-affine objects (1961)
- Cells grown on a lattice
- One single rule for growing the colony:

  – In each step, one of the lattice sites next to the populated areas is chosen randomly and occupied.

  - Or: in each time step, a randomly chosen (non-motile) bacterium proliferates.



- Primitive, but universal model

Eden-model

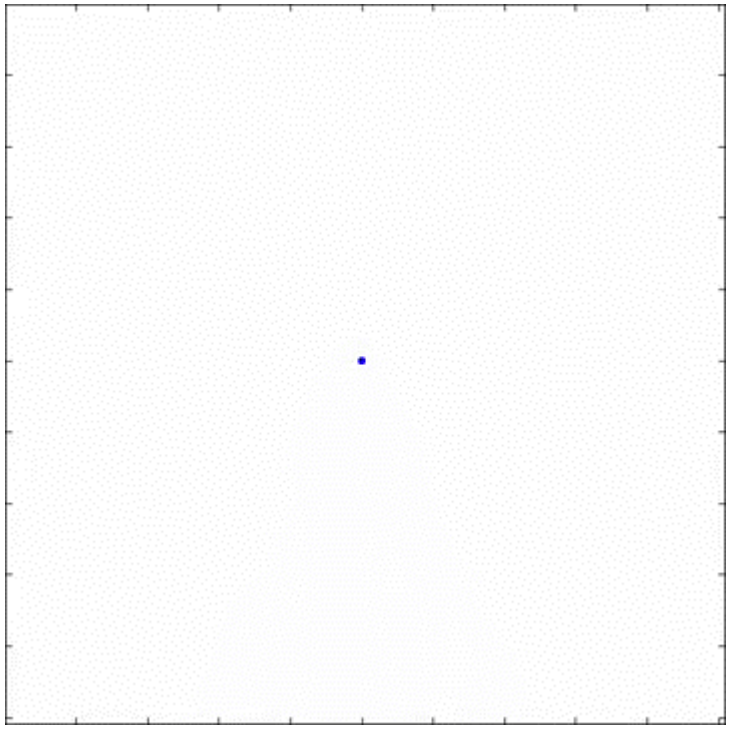
A typical colony in the Eden model grown on a strip of 256 lattice units.

- Initial step:

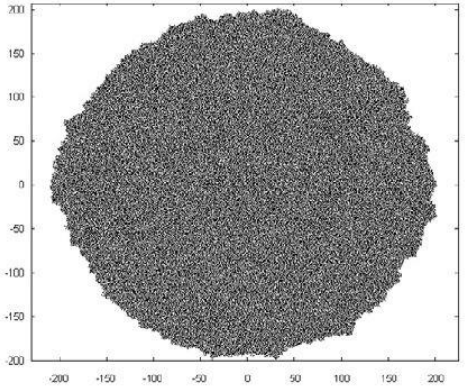
  1 occupied cell

- Variants:
  - Each position with same probability
  - Higher number of occupied neighbors increase the probability
- Variants of the model leave the statistical features of the developing clusters invariant in the asymptotic limit.

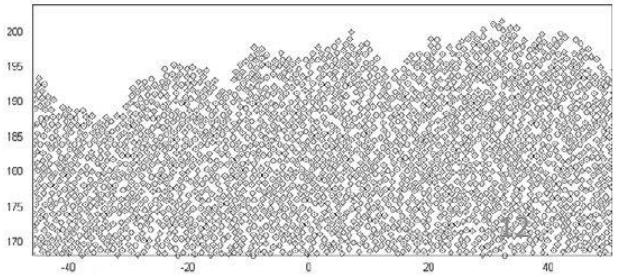
Simulations of the Eden model in 2D



- The lattice can destroy the rotational symmetry
- Continual model is more realistic



https://youtu.be/hluvLTwMFOs



Summary of the Eden model

- The surface contains “overhangs”
- Basic assumptions:
  - The units can not move (no “diffusion”)
  - Multiplication on the surface
- The model is simple but can be applied to many phenomena – “universality”
- The result is a self-affine surface
- KPZ model
  - The time evolution of the profile of a growing interface
  - Kardar, Parisi, Zhang: Dynamic scaling of growing surfaces. Physical Review Letters (1986)

The Kardar-Parisi-Zhang (KPZ) equation

$$\partial_t h = \nu \partial_x{}^2 h + \frac{\lambda}{2}(\partial_x h)^2 + u + \eta$$

- h : Height of the surface
- ∂_t : Partial derivative with respect to time
- ∂_x : Partial derivative with respect to the space coordinate *x*; ($\partial_x{}^2$: second derivative)
- ν : surface tension coefficient (*nu*)
- u : growth speed, perpendicular to the surface
- η : uncorrelated noise (stochastic)

The KPZ step-by-step

$$\partial_t h = \nu \partial_x{}^2 h + \frac{\lambda}{2}(\partial_x h)^2 + u + \eta$$

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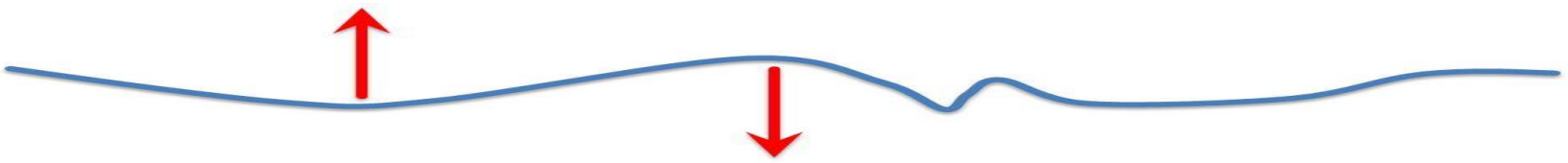
Speed of vertical growth:

$$\partial_t h(x,t)$$

Components:

1. Surface tension term $\nu \partial_x{}^2 h$
   - *2nd* derivative negative → local max (“top of a hump”)
   - *2nd* derivative positive → local min (“bottom of a swale”)
   - Tends to smoothen the interface
   - Does not permit discontinuities (large jumps) in *h*
   - ν: surface tension coefficient



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Speed of vertical growth: $\partial_t h(x,t)$

2nd component: makes the surface lumpy

$$\Delta h = \frac{u \cdot \Delta t}{\cos\varphi} = u \cdot \Delta t \left(\frac{1}{\cos\varphi}\right) = u \cdot \Delta t \sqrt{1 + tg^2\varphi} \approx$$

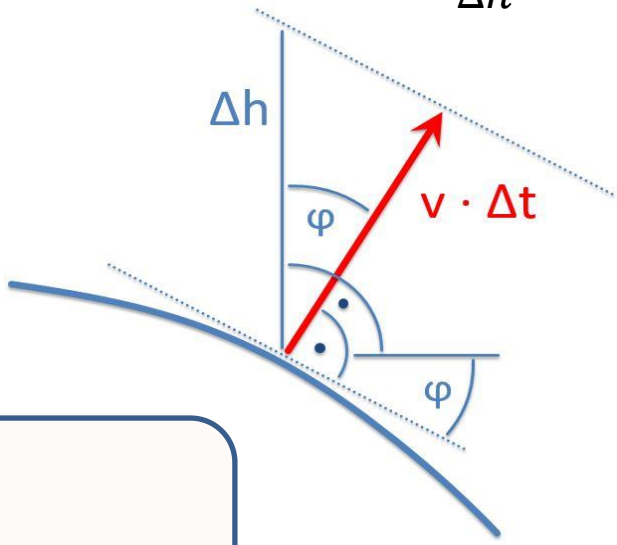
$$\approx u \cdot \Delta t \left(1 + \frac{tg^2\varphi}{2}\right) = u \cdot \Delta t + \frac{u \cdot \Delta t}{2} tg^2\varphi \approx$$

$$\approx u \cdot \Delta t + \frac{u \cdot \Delta t}{2} (\partial_x h)^2$$

During a small Δt period of time the growth of the surface:

$$\frac{\Delta h}{\Delta t} \approx u + \frac{u}{2}(\partial_x h)^2$$

$$\cos\varphi = \frac{u \cdot \Delta t}{\Delta h}$$



Due to other effects $\frac{u}{2} \rightarrow \frac{\lambda}{2}$ (more general equation)

1D→2D

- $x \rightarrow$ r
- $\partial_x \rightarrow \vec{\nabla}$

$1 + tg^2 x = \frac{1}{cos^2 x}$;

if ε<<1, then $\sqrt{1+\varepsilon} \approx 1 + \frac{\varepsilon}{2}$

if $\varphi \ll 1$, then tg $(\varphi) \approx \partial_x h$

The KPZ step-by-step 1D→2D

$$\partial_t h = \nu \partial_x{}^2 h + \frac{\lambda}{2}(\partial_x h)^2 + u + \eta$$

- h : Height of the surface
- ∂_t : Partial derivative with respect to time
- ∂_x : Partial derivative with respect to the space coordinate *x*; ($\partial_x{}^2$: second derivative)
- ν : surface tension coefficient (*nu*)
- u : growth speed, perpendicular to the surface
- η : uncorrelated noise (stochastic)

$$\partial_t h(\vec{r},t) = \nu \cdot \vec{\nabla}^2 h(\vec{r},t) + \frac{\lambda}{2}\left(\vec{\nabla} h\right)^2 + u + \eta(\vec{r},t)$$

- Smoothening component (surface tension)
- Roughening
- noise: $\eta = \eta(\vec{r},t)$: stochastic (=non-deterministic), uncorrelated in space and time

Comments:

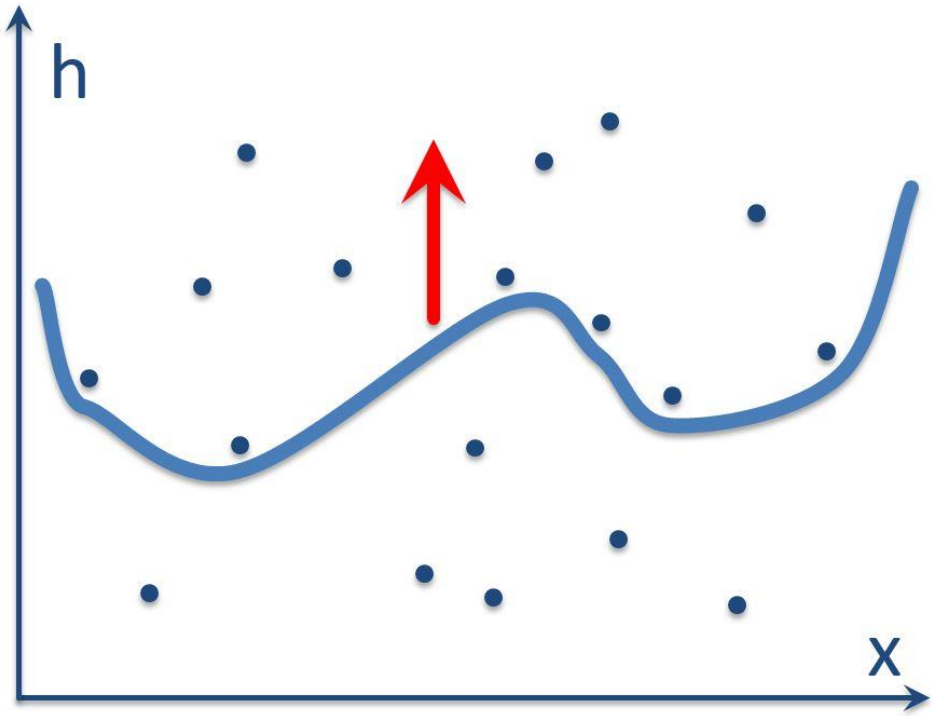
- In case of uncorrelated $\eta(\vec{r},t)$ noise the resulting surface is self affine
- In this case (and in the Eden model) the roughness exponent H=1/2, in contrast to experiments, where H≈0.7, …, 0.8
- Reason: in the KPZ the noise is uncorrelated in time (↔ reality!)

KPZ with quenched noise

- Uncorrelated noise (in time):
  - If the noise is $\eta(\vec{r}, t)$ at the position $\vec{r}$ at time t, then the noise is "*independent*" of $\eta(\vec{r}, t)$ at the same place, at time $t + \Delta t$.
- In other words:
  - If the spreading of the colony sticks at time t at position $\vec{r}$ due to the local inhomogeneity $\eta(\vec{r}, t)$ of the surface (gel), then at the same position, Δt later, the noise would be independent (uncorrelated), that is, the surface would move on.
- In contrast, the reality is that
  - Such noises are often constant in time
  - The colony moves in an inhomogeneous medium, in which the inhomogeneity is constant in time
  - The noise "quenches" into the medium. "quenched noise"

KPZ with quenched noise

- If the noise is constant (and fixed) in time:
  - If the spread of the colony surface sticks at a given point $\vec{r}$, then this “halt” can be extensive in time, since the media does not change.
  - Results in a surface proceeding in a hoping/jiggling manner (points are blocks).



KPZ with quenched noise

- Defining the $\eta(\vec{r}, t)$ quenched noise:
  - Let us consider a *Δ(u)* function with the following properties:
    - If *u* is close to 0, then $\Delta(u) \cong 1$ (in a small, finite interval), and
    - Everywhere else *Δ(u)*=0.
    - a "blurred" Dirac-delta
  - $\eta(\vec{r}, t) := 2D\tilde{\eta}(\vec{r}, h(\vec{r}, t))$
    - $\tilde{\eta}$ is normalized noise
    - whose spatial autocorrelation is $C_{\tilde{\eta}}(\vec{r}, \overrightarrow{r'}) = \Delta(|\vec{r}|)\Delta(|\overrightarrow{r'}|)$
      - That is, correlated in a very small spatial interval
    - *D* : average magnitude of the noise as $\sqrt{C_{\tilde{\eta}}(0,0)} = \sqrt{2D}$
  - We incorporate this quenched noise into the KPZ, we get:

$$\partial_t h(\vec{r}, t) = \nu \cdot \vec{\nabla}^2 h(\vec{r}, t) + \frac{\lambda}{2}\left(\vec{\nabla} h\right)^2 + u + \eta(\vec{r}, h(\vec{r}, t))$$

KPZ with quenched noise

$$\partial_t h(\vec{r},t) = \nu \cdot \vec{\nabla}^2 h(\vec{r},t) + \frac{\lambda}{2}\left(\vec{\nabla} h\right)^2 + u + \eta(\vec{r}, h(\vec{r},t))$$

- By "appropriate" choice of the time and length units the parameters λ, ν and u can be transformed out
  - the $\lambda=\nu=u$ case:

$$\partial_t h = \vec{\nabla}^2 h + \frac{1}{2}\left(\vec{\nabla} h\right)^2 + 1 + \eta = \vec{\nabla}^2 h + \sqrt{1 + \left(\vec{\nabla} h\right)^2} + \eta$$

(where the magnitude of η is $\sqrt{\eta\eta} = \sqrt{C_{\tilde{\eta}}(0,0)} = \sqrt{2D}$)

- Two extreme cases:
  1. $D \ll D_* \sim 1$
  2. $D > D_* \sim 1$

KPZ with small quenched noise

- First Case: $D \ll D_* \sim 1$
  - The interface is never pinned, advances with a steady velocity
  - Fluctuating noise with some finite temporal correlations
  - The standard KPZ can be applied
  - Resulting interfaces with H=1/2.
  - Experimental support: Colonies grown on soft agar gel (small pinning effect) showed standard KPZ-like behavior with surface characterized by H=1/2.

KPZ with big quenched noise

- Second Case: $D > D_* \sim 1$
  - The interface is pinned at some certain points, for an extended period of time (until the neighboring segments pull it out)
  - If the density of the pinning points is high enough, then the propagation of the whole surface can be blocked.
  - The shape of the frozen colony is determined by the distribution of these pinning sites (and independent of the growth dynamics).

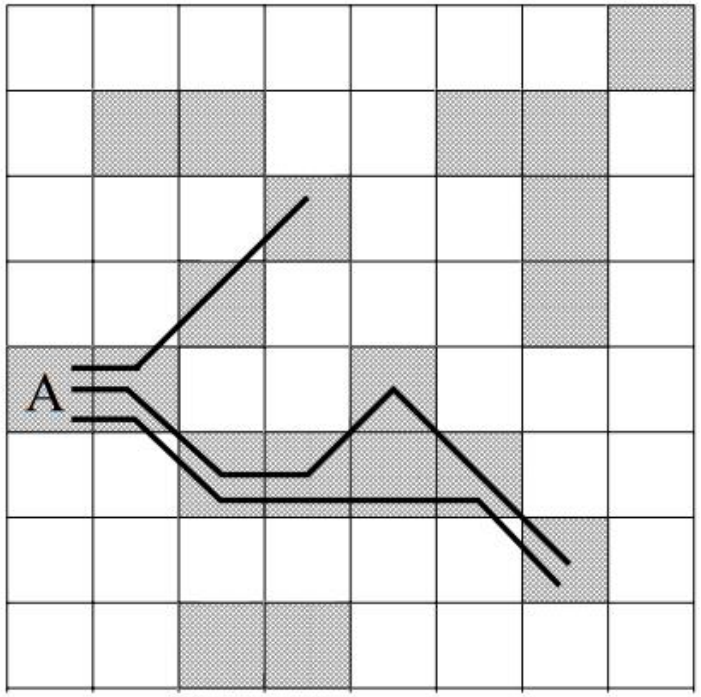
➢ The surface roughening can be mapped onto a **directed percolation problem**:

➢ finding directed and connected paths

➢ Let us consider a **lattice** instead of the continuous case (discrete model, regarding both *h* and the location *(x,y)*)

Directed percolation

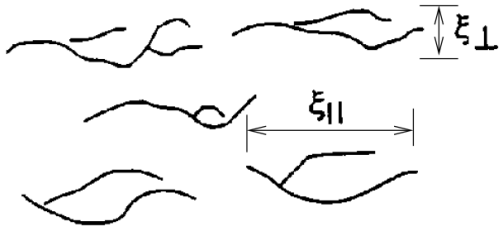
1. Let us define each lattice site as
2. “pinning” with a probability $0<p<1$. (gray squares)
3. We start from one end of the panel
4. On the pinning sites we can move ahead, up and down (but not backwards)
5. Do we reach the other end of the board?

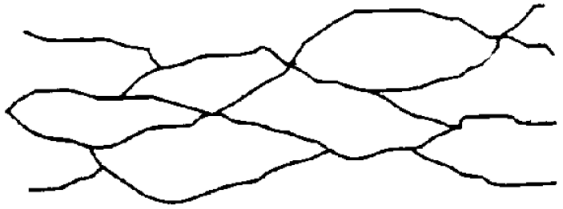


- The chain of the pinning sites define a directed percolation cluster (if it exists).
  - Complete blocking of the interface propagation appears when there is a directed, connected path (a directed percolation cluster)
  - The propagation stops along these clusters

Correlation lengths of directed percolation clusters

- DPC is characterized by two correlation length:
  1. Parallel to the interface (to the preferred direction) $\xi_{\parallel}$
  2. Perpendicular to the interface (to the preferred direction) $\xi_{\perp}$





- There is a critical probability p_c (defining the density of the pinning sites)

$$\xi_{\parallel} \sim |p - p_c|^{-\nu_{\parallel}} \quad \text{and} \quad \xi_{\perp} \sim |p - p_c|^{-\nu_{\perp}}$$

with

$$\nu_{\parallel} = 1.733 \text{ and } \nu_{\perp} = 1.097 \quad \text{(numerical results)}$$

- The width of the interface: $w \cong \xi_{\perp}$
- Complete blocking of the interface when $\xi_{\parallel}$=L

(L is the system size)

- The width of the interface at the critical point:

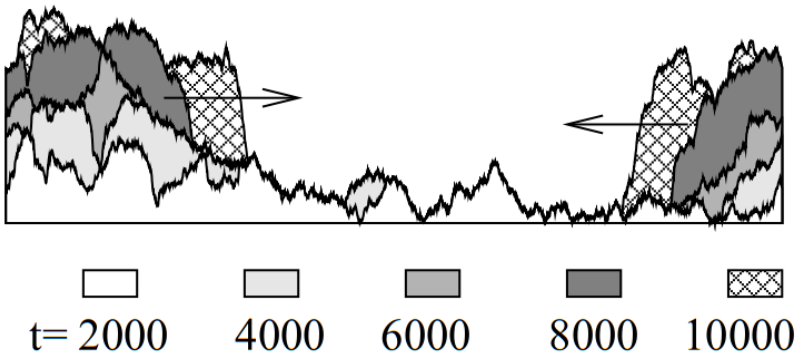
$$L^{H} \sim w \cong \xi_{\perp} \sim |p - p_c|^{-\nu_{\perp}} \sim \xi_{\parallel}^{\frac{\nu_{\perp}}{\nu_{\parallel}}} \approx L^{\frac{\nu_{\perp}}{\nu_{\parallel}}} \quad \rightarrow \quad \boxed{H = \frac{\nu_{\perp}}{\nu_{\parallel}} = 0.633}$$

Directed percolation

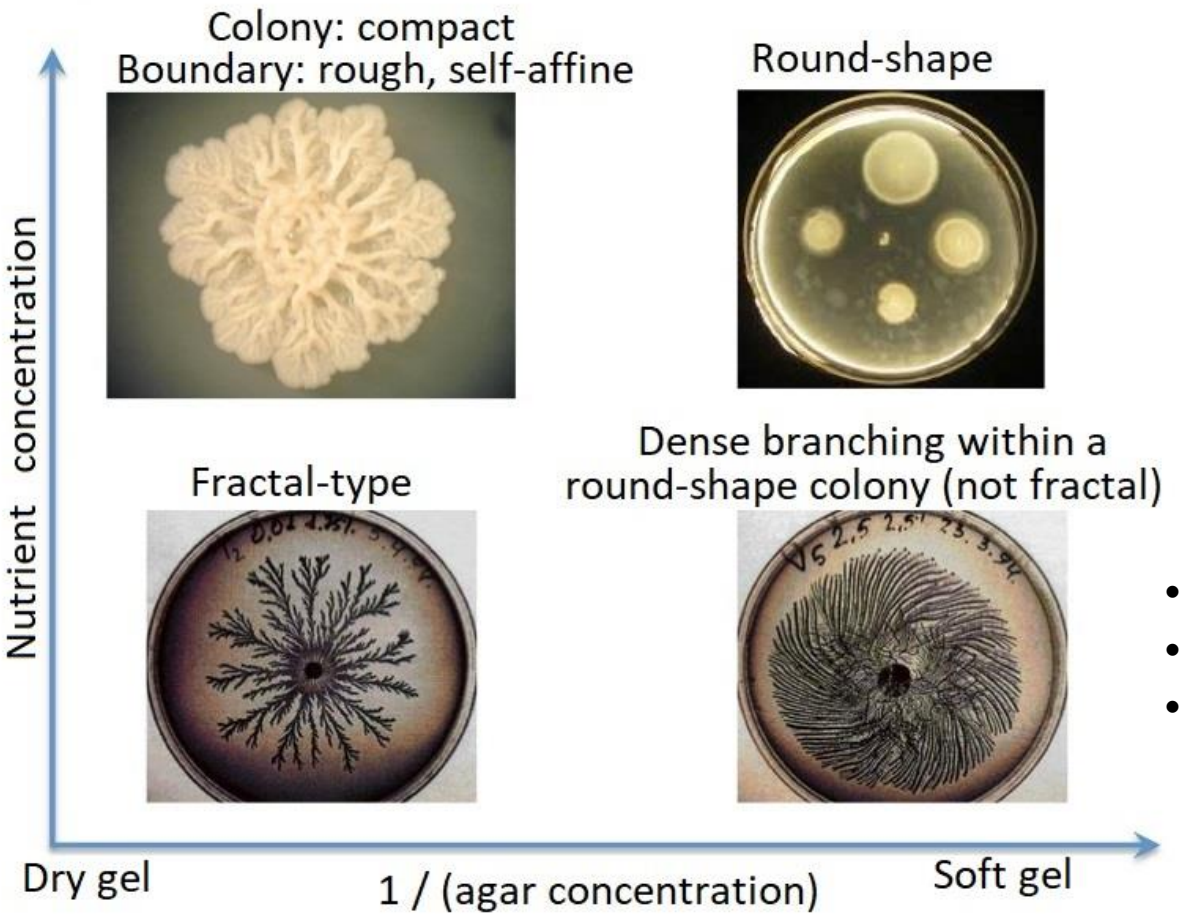
- The numerical result for the (discrete) directed percolation problem is H=0.633
  - (complete blocking)
- Experimental results: H≈0.7-0.8
- Reason: the observed colonies have both blocked and freely moving parts → *higher* roughness exponent (H) than for the blocked interface.
  - Numerical simulations:

    H≈0.71-0.75

    (close to the observations)
- KPZ with quenched noise and the DP simulations have the same results



Branching morphology

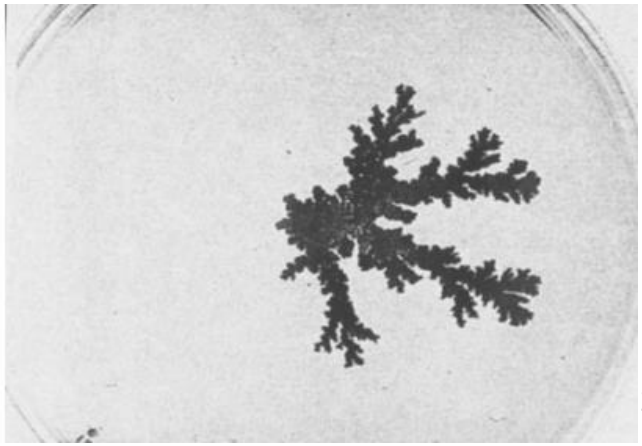


Baillus subtilis colony, under nutrient-poor conditions. 8 and 19 days after inoculation.

- Nutrient-poor agar substrate
- Complex, branching colonies
- Not exhibited by all strains (but by many)

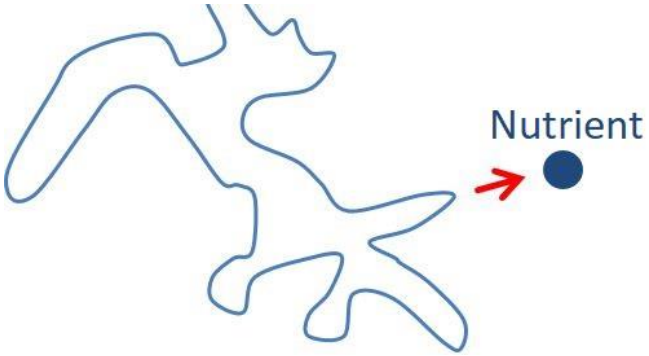
Branching morphology – colony formation

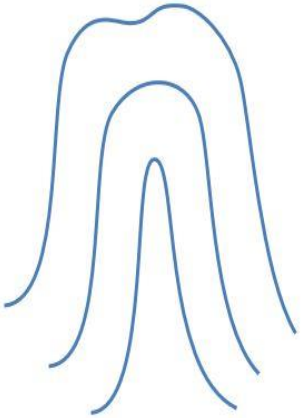
- Basic assumption:
  - the growth of the colony is *diffusion-limited*:
  - The multiplication of the bacteria is determined by the locally available nutrient
    - At the beginning: local nutrient is enough to maintain the growth
    - After some bacterial multiplication, nutrient deprivation progresses in and around the colony
    - Further growth depends on the diffusive transport from distant regions of the petri-dish
  - Experimental support
    - Non motile *B. subtilis* grows only towards nutrient-rich regions



Branching morphology – colony formation

- The speed of the growth is determined by the nutrient diffusion
- The colony develops towards the nutrient
- Instability:
  - Due to some random perturbation a small part of the colony advances “ahead” (towards some nutrient)
  - This part of the colony gets closer to the nutrient
  - Can multiply faster
- This process stops at a certain curvature
  - Certain amount of neighboring cells are needed
  - A certain “steady shape” is set
- New perturbation: new branch

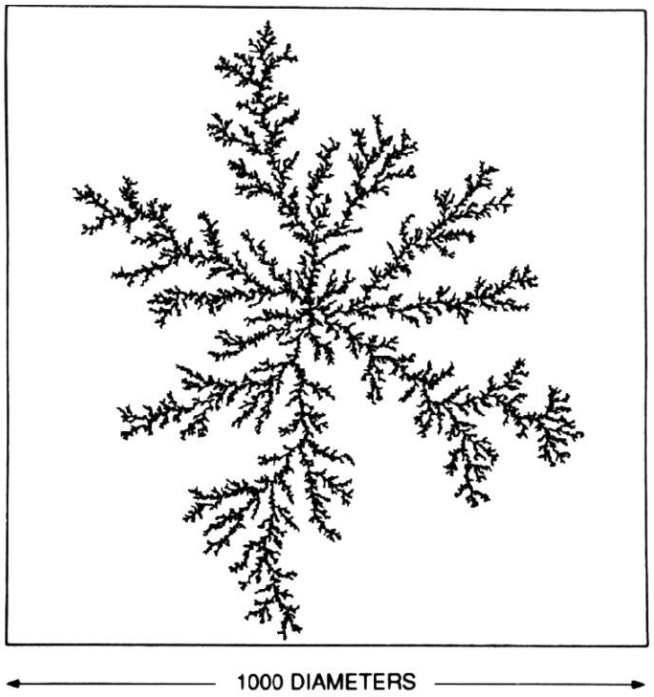




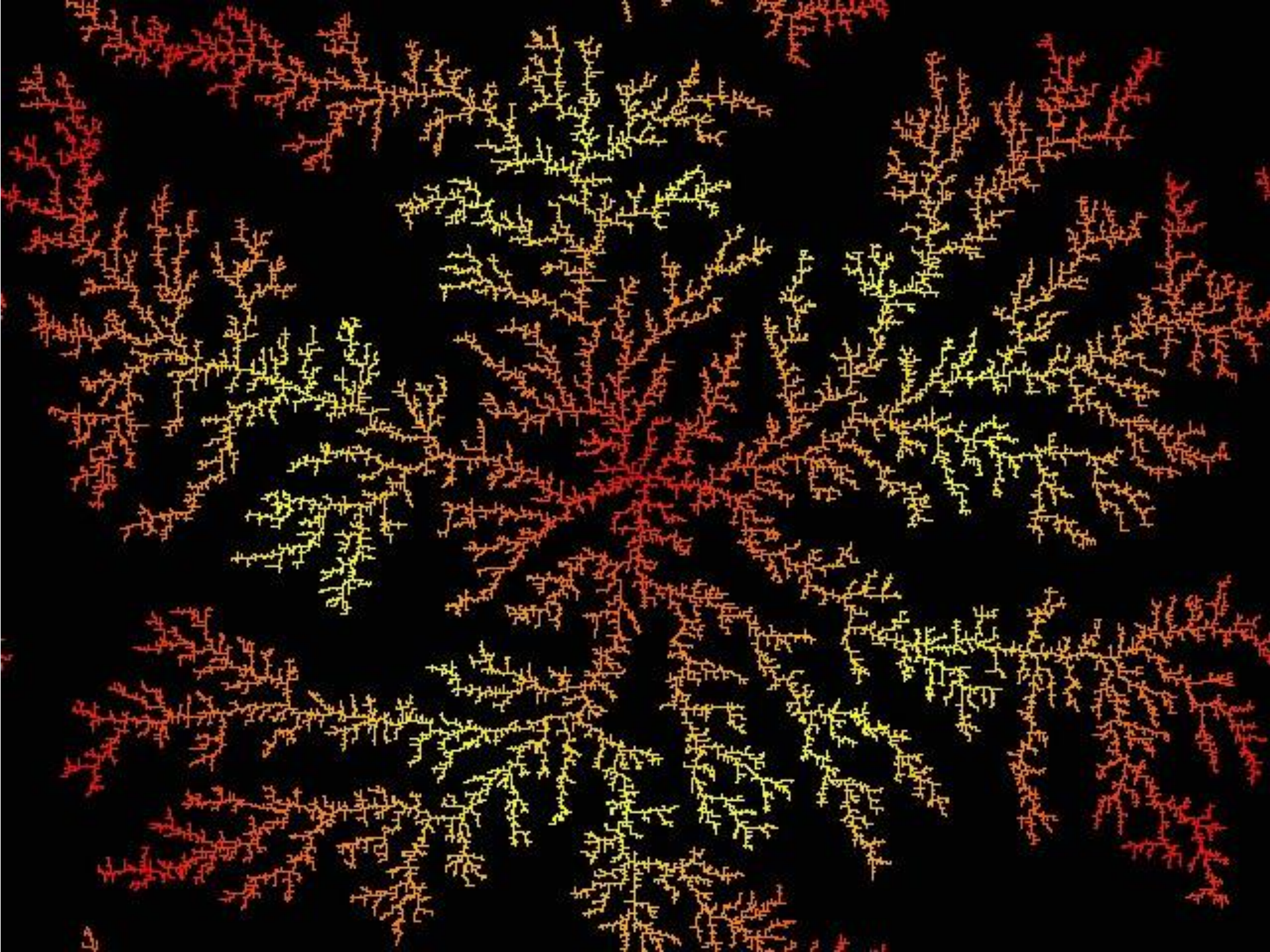
Diffusion-Limited Aggregation (DLA)

- The definition of the basic DLA algorithm:
  - Start: 1 cell
  - In each time step:
    - A particle (performing random walk) departs from infinity

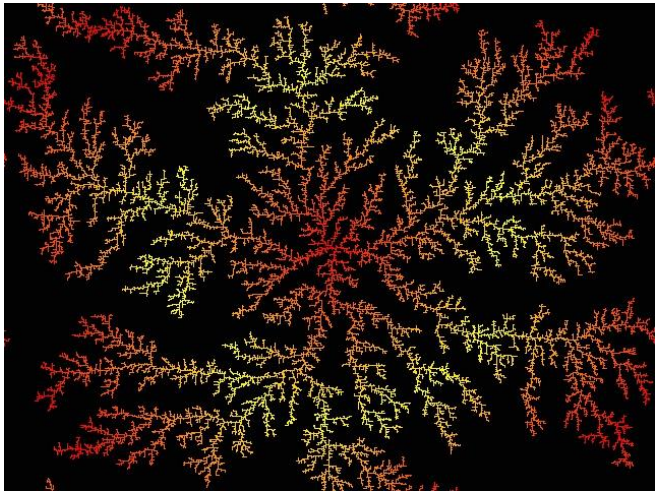
      (in simulations from finite distance)
    - Sticks to the colony upon graze
- Result: Fractal-type clusters

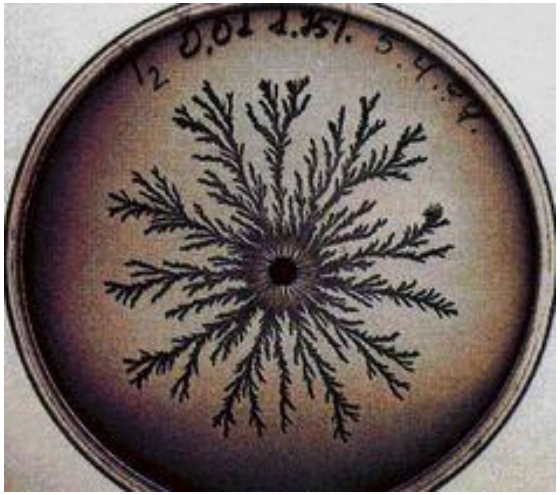


Typical DLA cluster with 50, 000 particles



Relation to bacterium colonies





- Random walk of the particle ~ diffusion of the nutrient
- Sticking to the colony ~ bacterium proliferation

- Non-motile bacteria!
- Very simple model (1 “nutrient-unit” = 1 multiplication) generating realistic formations → “universality”

Refinement of the DLA model – Modeling non-motile bacteria

- Assumptions:
  - Bacteria interact with each other
  - Each particle (cell) is characterized by
    - Space coordinate x_i
    - Energy state E_i (or cell cycle state)
      - $E_i < 0$: spore state. Without nutrient, remains in this state
      - $0 < E_i < 1$: right after multiplication
      - $E_i > 1$: has enough energy to multiply
- Notations:
  - ω_i : nutrient consumption rate
  - κ : conversion factor relating the maximal nutrient consumption rate with the shortest cell cycle time

    (nutrient → energy conversion)
  - ϵ : generic "maintenance" term (not directly contributing to growth)

The energy-level of bacterium *i*: uptake - consumption

$$\frac{dE_i}{dt} = \kappa \cdot \omega_i - \epsilon$$

Modeling non-motile bacteria – limits of the nutrient uptake

- Further notations:
  - ω_{max} : maximal nutrient uptake rate of the cells
  - κ : efficiency of the enzymatic reaction converting the nutrient into energy
  - $c(x_i)$: nutrient concentration (around cell *i*)
  - $\rho(x_i)$: local cell density
  - $\omega_0 c$: maximal diffusive transport from the substrate to the cell
  - ω_i : nutrient consumption rate (of bacterium *i*)
- The rate with which the cell-mass grows:

$$\rho(x_i)\omega_i = min[\omega_{max}\rho(x_i), \omega_0 c(x_i)]$$

→ The nutrient-uptake is limited by the enzymatic rates and local nutrient concentration (the maximal speed with which cell *i* can take in the nutrient)

Modeling non-motile bacteria – How the local nutrient concentration varies

- Bacteria use up the nutrient
- Changes in c are given by the diffusion equation with the appropriate sink terms at the position of the active particles:

$$\frac{dc}{dt} = D_c \nabla^2 c - \sum_i \omega_i \delta(x - x_i)$$

Diffusion

Sinks: the cell at location x_i consumes the nutrient with rate ω_i

Summary: non-motile bacteria in nutrient-poor environment

(i) The energy-level of cell *i*

$$\frac{dE_i}{dt} = \kappa \cdot \omega_i - \epsilon$$

(ii) Cell-mass growth rate

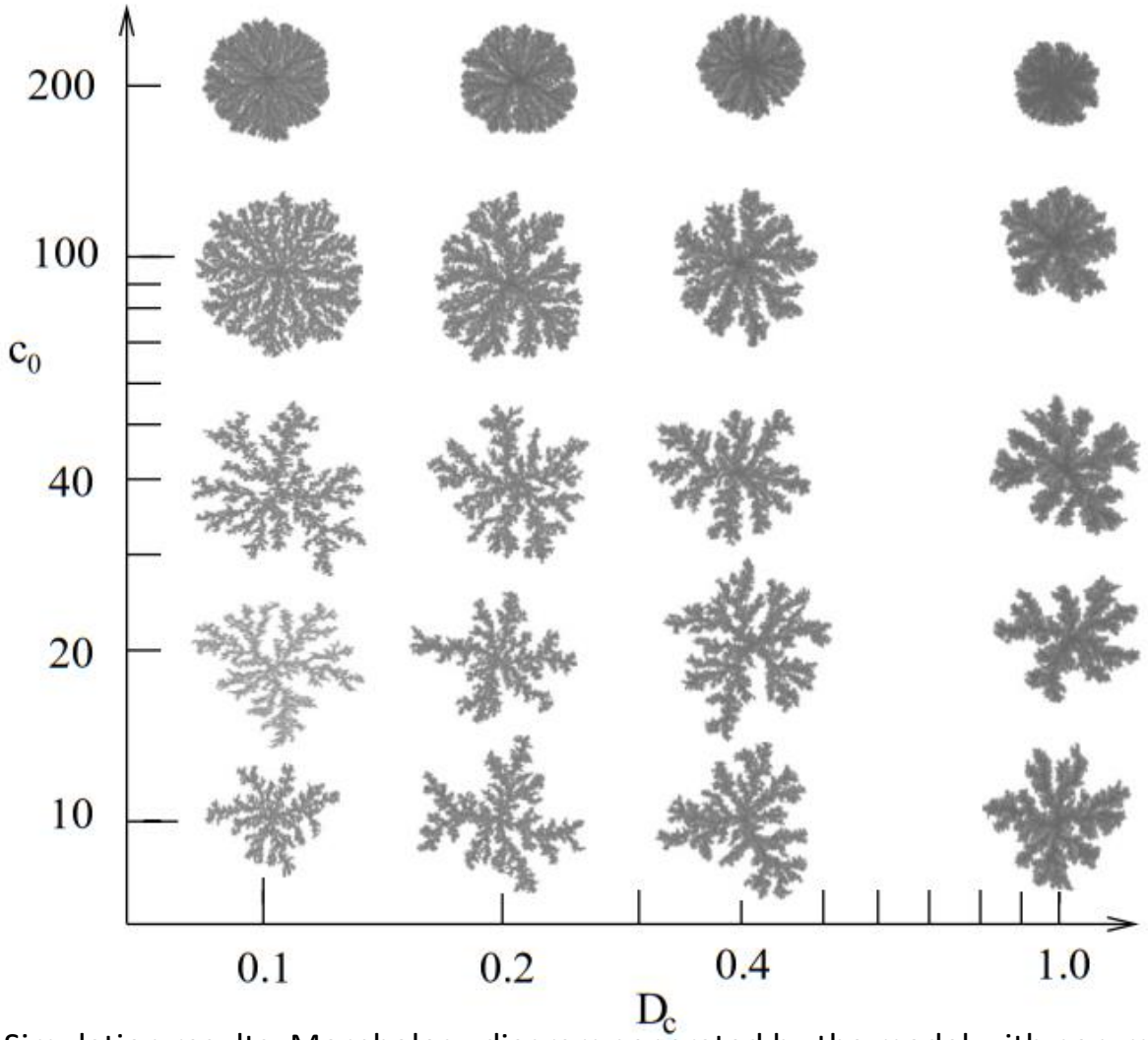
$$\rho(x_i)\omega_i = min[\omega_{max}\rho(x_i), \omega_0 c(x_i)]$$

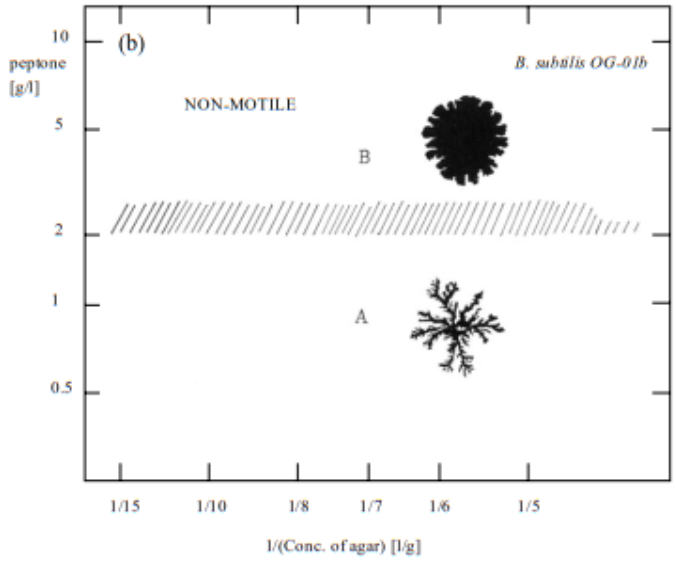
(iii) Changes of the local nutrient concentration

$$\frac{dc}{dt} = D_c \nabla^2 c - \sum_i \omega_i \delta(x - x_i)$$

- E_i : energy level of cell *i*
- κ : efficiency of the enzymatic reaction converting the nutrient into energy
- ω_i : nutrient consumption rate
- ϵ : generic "maintenance" term (not directly contributing to growth)
- $\rho(x_i)$: local cell density
- ω_{max} : maximal nutrient uptake rate of the cells
- $c(x_i)$: nutrient concentration (around cell *i*)
- $\omega_0 c$: maximal diffusive transport from the substrate to the cell
- D_c : nutrient diffusivity

Results: Modeling non-motile bacteria with the refined DLA model





Related experiments: the morphology diagram of the non-motile B. subtilis OG-01b strain

Simulation results: Morphology diagram generated by the model with non-motile particles as a function of the initial nutrient concentration (c_0)and nutrient diffusivity (D_c). The colonies were grown (in the computer) until either their size or the number of bacteria reached a threshold value.

Motile bacteria with the DLA model

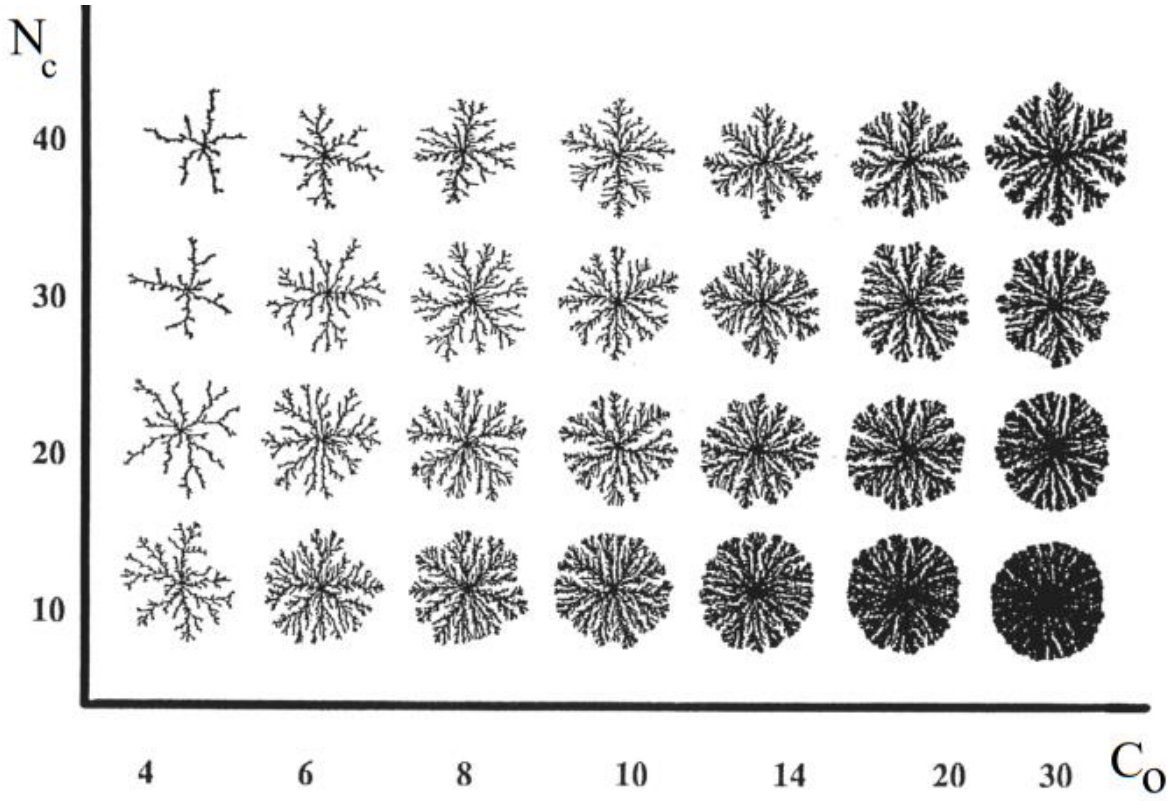
- Rules (i)-(iii) remain the same
  - (i) energy-level of cell *i*
  - (ii) cell-mass growth rate
  - (iii) changes of the local nutrient concentration
- Non-motile cells → self-affine surface
- Motile bacteria → smooth surface. But they still can not migrate on dry agar surface, only where some surfactant was secreted:
- New rules:
  - (iv) The active particles move randomly (with Brownian motion) within a boundary:

$$\frac{dx_i}{dt} = v_o \vec{e}$$

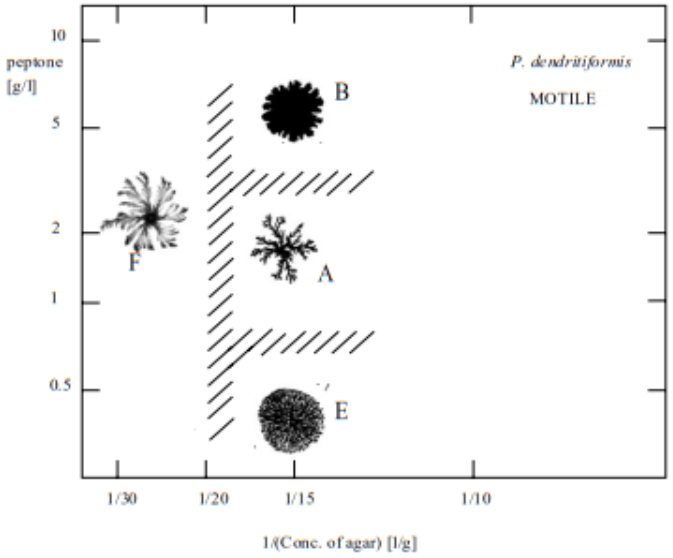
where $\vec{e}$ is a unit vector pointing in a random direction

  - (v) The propagation of the bacteria is assumed to be proportional to the local density of the active cells. Collisions of the particles with the boundary is counted, and when a threshold value (N_c) is reached, the neighboring cell is occupied as well. (the boundary shifts forward)

Results: Modeling motile bacteria with the refined DLA model (on hard agar gel)



Morphology diagram generated by the model with motile bacteria as the function of the initial nutrient concentration (C_o) and agar gel "hardness", (the threshold value N_c for the colony borderline displacement).



Corresponding experimental results: Morphology diagram of *Paenibacillus dendritiformis*. Agreement with the model results within a limited region of the parameters, but it fails to predict the formation of the thin, straight radial branches at very low food concentrations.

Simulation results:

- Nice agreement with the experiments, but
- Fails to explain the transition between the

Fractal-type and non-fractal-type colonies

Solution: assuming repulsive chemotaxis signaling among the cells. Due to the repulsion the cells by-pass each other: the random Brownian motion becomes biased.

Simulation results without (a) and with (b) repulsive chemotaxis signaling.