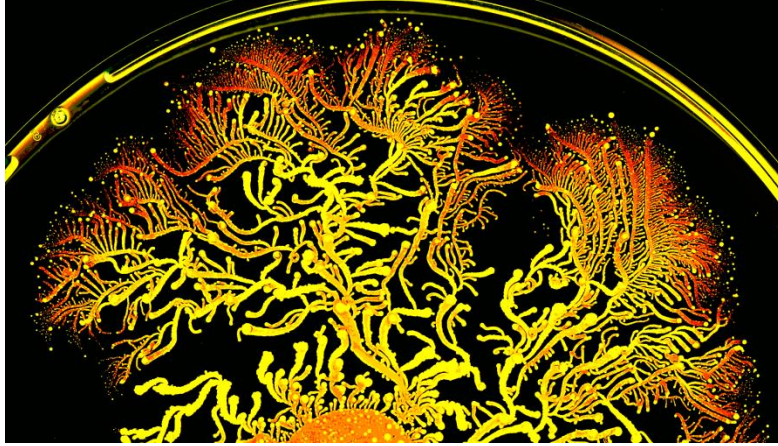
The geometry of bacteria colonies I

The microbiological background of motion, morphology diagram, Fisher equation and the origins of instability

October 2, 2017

Why exactly bacteria colonies?



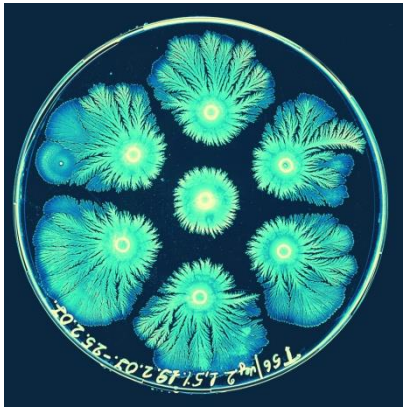
Colony of *Paenibacillus* vortex bacteria

From a more quantitative point of view

- A system with interactions that are simple enough to be captured by quantitative models
- A system whose collective behavior can be explored with computational models
- They can give an insight into the formation of self-organized biological structures
- Also, an example for how biological systems can be described with tools borrowed from physics

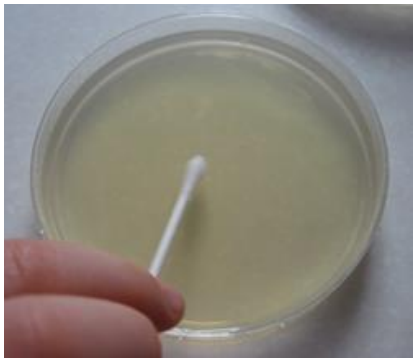
Why exactly bacteria colonies?

An easy-to-study case for biological self-organization



- Unicellular organisms
- Living in colonies
- They are easy to handle in experiments
- Simple and understood interaction rules
- They produce various spatio-temporal patterns
- The patterns are often independent of the interaction details - “universality”
- Dependency on environmental conditions
- Experiments can be reproduced
- Theories can be modeled and tested via computer simulations

The set-up of the simplest experiments for colony formation



Bacteria are grown on the surface of agar gel (an alga)

- "Dry" surface (= big agar concentration)
  - The cells can not move (to spread over the substrate can take even weeks)
  - The duplication time is much smaller

    → *proliferation* is the key factor determining the morphology
- "Soft" gel (= small agar conc.)

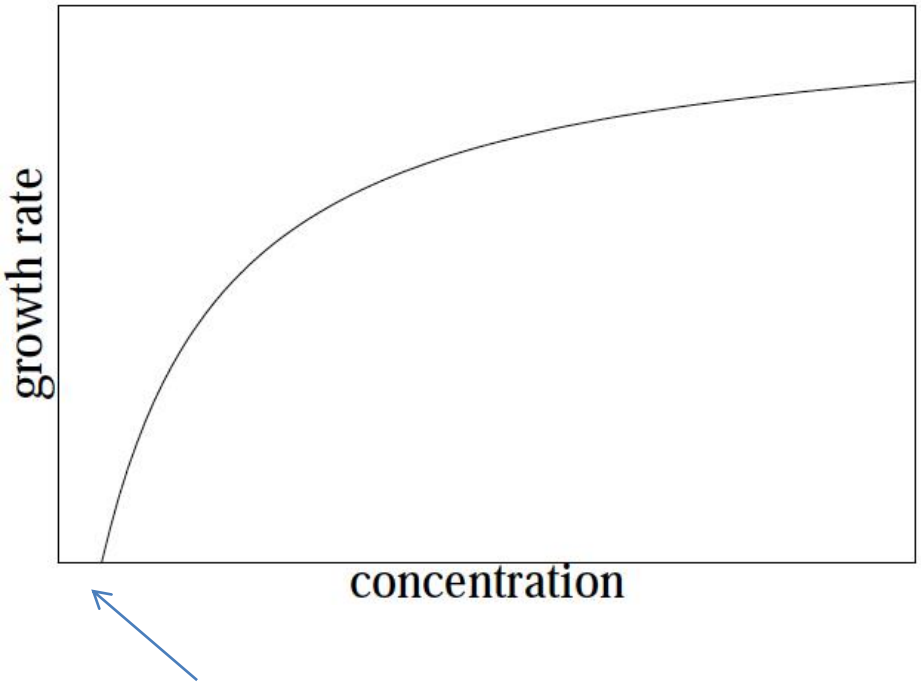
  Or: the bacteria produce surfactant
  - The colony spreads over the substrate in a few hours

    → *bacterial motion* and *chemotaxis* are the main factors

Microbiological background - Proliferation

- Growth (the increase of the number and total mass of bacteria) strongly depends on the nutrient concentration

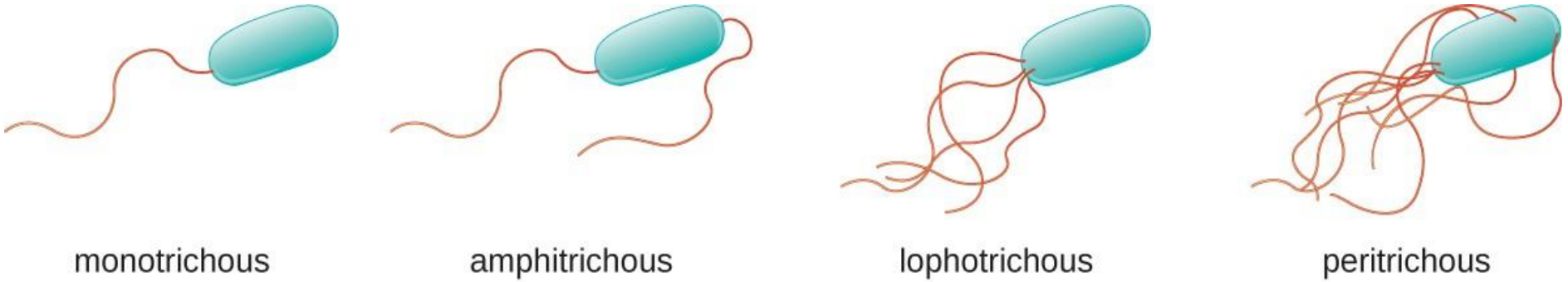
- Rate of growth (number of cell divisions within a population of unit size during a unit time interval) increases with the nutrient concentration in a hyperbolic manner.



A certain amount of nutrient is required to maintain the intracellular biochemical processes

Microbiological background - Motility

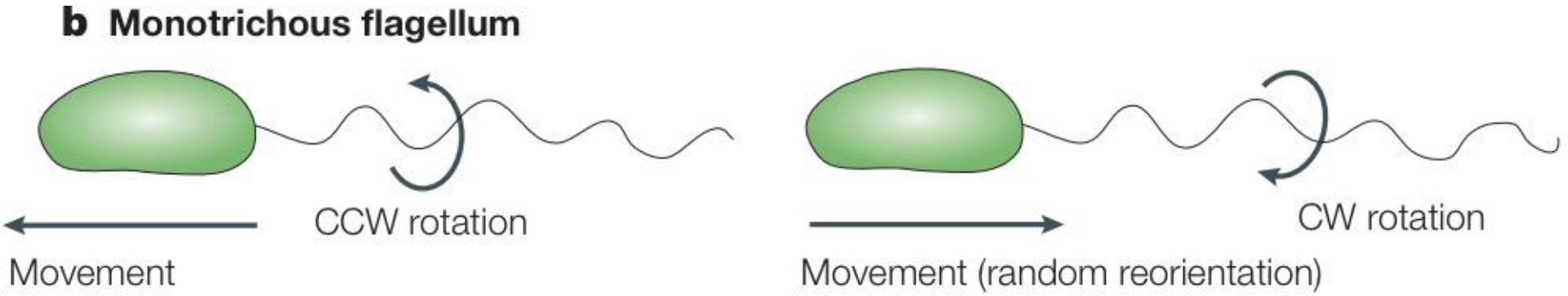
Procaryotes move in aquatic environment by rotating their flagella



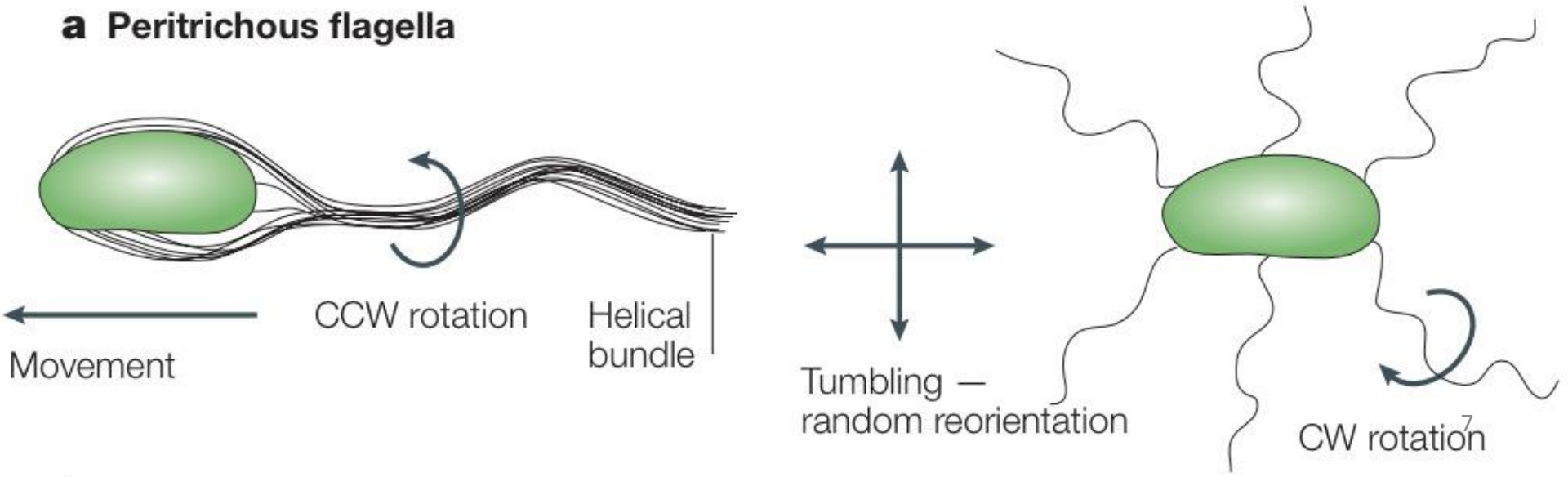
Bacteria can have

- One flagellum, “monotrichous”
- A pair of flagella at the opposite cell poles, “amphitrichous”
- Clusters of flagella at the poles, “lophotrichous”
- Uniformly distributed flagella over the cell membrane, “peritrichous”

The direction of flagellar rotation determines the motion



The forward motion is interrupted by short intervals of “tumbling”



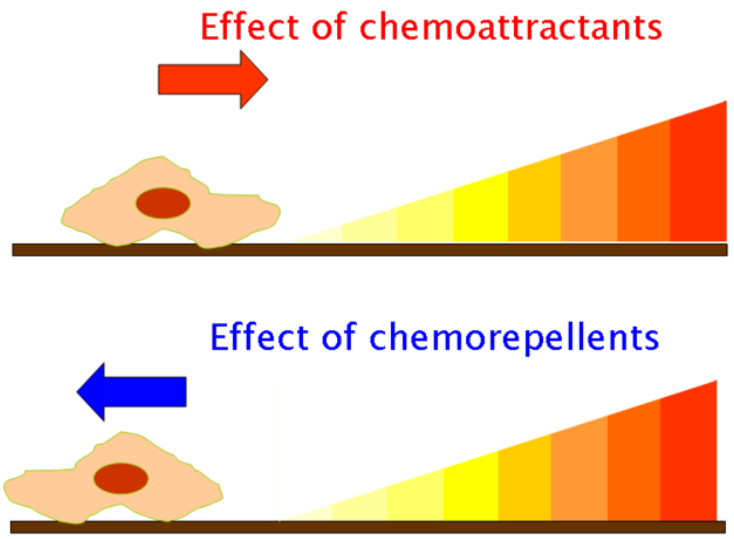
Bacterial Motility - Gliding

- Entirely different type of motility (flagella-independent)
- Slower and smoother than swimming
- Requires surface contact
- Employed by many strains when moving on surfaces
- No visible cellular structures associated – little is known about it
- Slime secretion
- Motion types varies greatly → probably more than one mechanisms exist
  - Gliding along the direction of the long axis of the cell (e.g. Myxococcus or Flexibacter)
  - Screw-like motion (e.g. Saprospira)
  - Direction perpendicular to the long axis (Simonsiella)

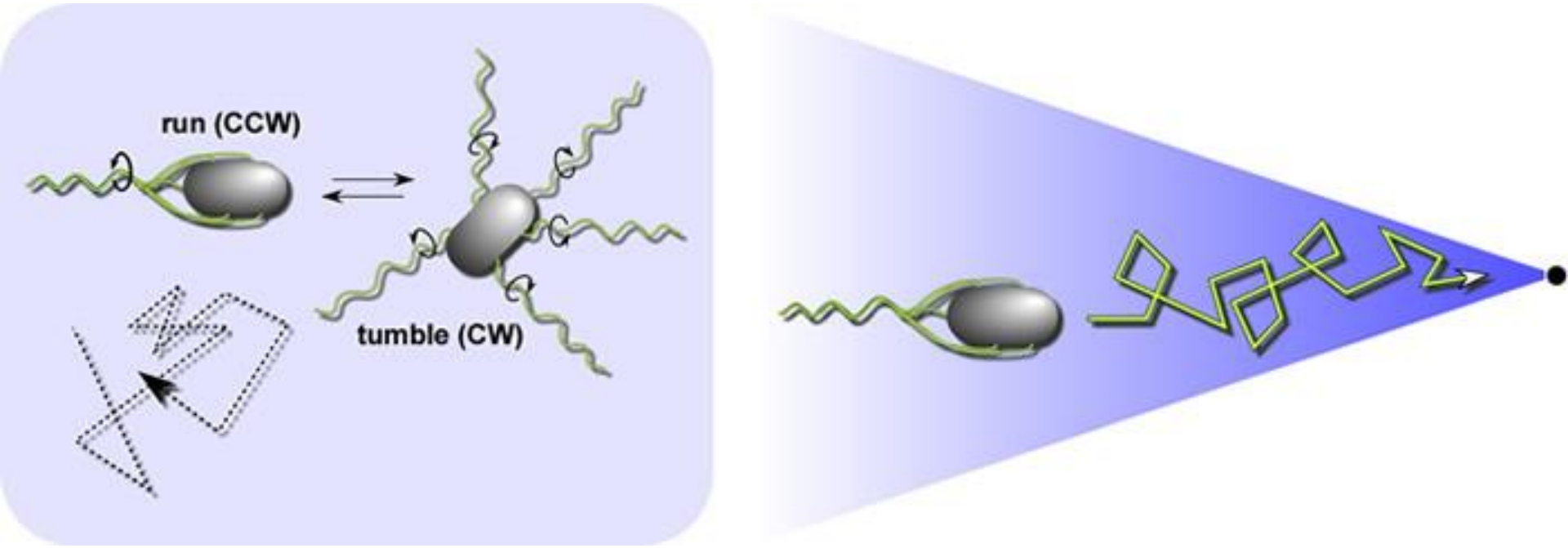
Microbiological background - Chemotaxis

- Bacteria are attracted by nutrients (sugar, amino acids, etc.) and repelled by harmful substances and metabolic waste products.
- Other environmental factors, e.g. temperature, light, oxygen concentration

- Stochastic process: chemical gradients modulate the tumbling frequency: repressed when moving towards chemoattractants
- A molecular machinery compares the changes of the chemical concentration *in time*.



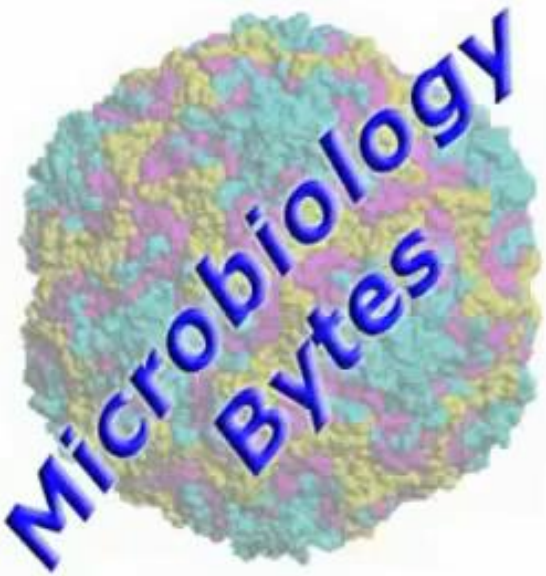
Microbiological background - Chemotaxis



Random and biased walks. **Left**: A random walk in isotropic environments. When the cell's motors rotate CCW, the flagellar filaments form a trailing bundle that pushes the cell forward. When one or more of the flagellar motors reverses to CW rotation, that filament undergoes a shape change (owing to the torque reversal) that disrupts the bundle. Until all motors once again turn in the CCW direction, the filaments act independently to push and pull the cell in a chaotic tumbling motion. Tumbling episodes enable the cell to try new, randomly-determined swimming directions. **Right** A biased walk in a chemo-effector gradient. Sensory information suppresses tumbling whenever the cell happens to head in a favorable direction. The cells cannot head directly up-gradient because they are frequently knocked off course by Brownian motion.

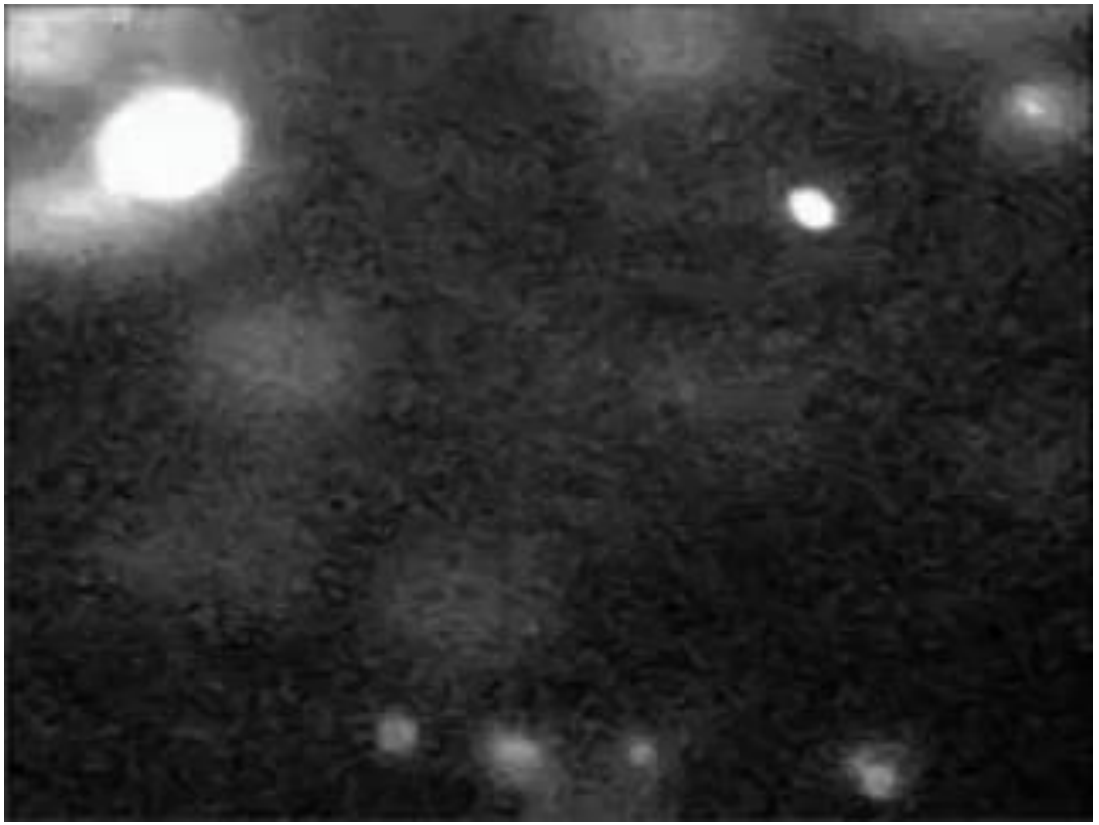
Source: http://chemotaxis.biology.utah.edu/Parkinson_Lab/projects/ecolichemotaxis/ecolichemotaxis.html

Microbiological background: Bacterial Motility. 4:35 mins



https://www.youtube.com/watch?v=zlFRJftA2bU

Bacterial Flagellum. 5:30 mins



https://www.youtube.com/watch?v=v1NnMmw8v80

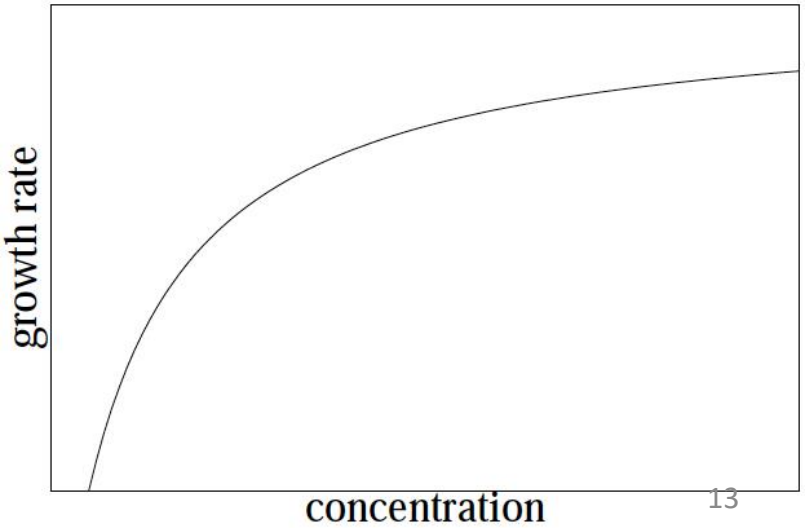
Morphology diagram

- Various environmental parameters influence colony development:
  - temperature, humidity, chemical composition of the substrate, etc.
  - Can result in different morphologies even for the same strain
- Most systematic experiments explore the relation between the concentration of the *agar* and *nutrients*.
- Characteristic colony shapes are assigned to the parameter pairs
- Agar concentration:

  consistency of the gel →
  - motility of the bacteria and
  - diffusibility of the nutrient
- Nutrient concentration:

  determines the

  proliferation rate



Morphology diagram of *Bacillus Subtilis*

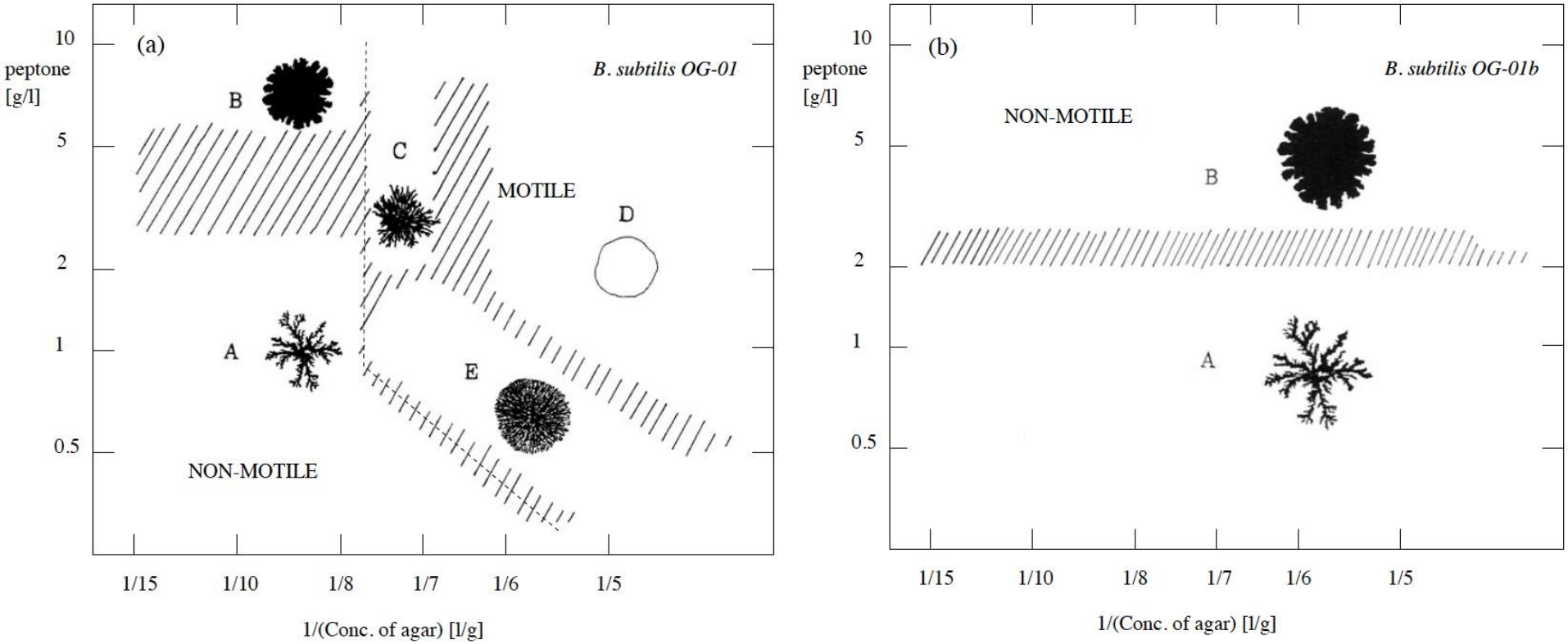
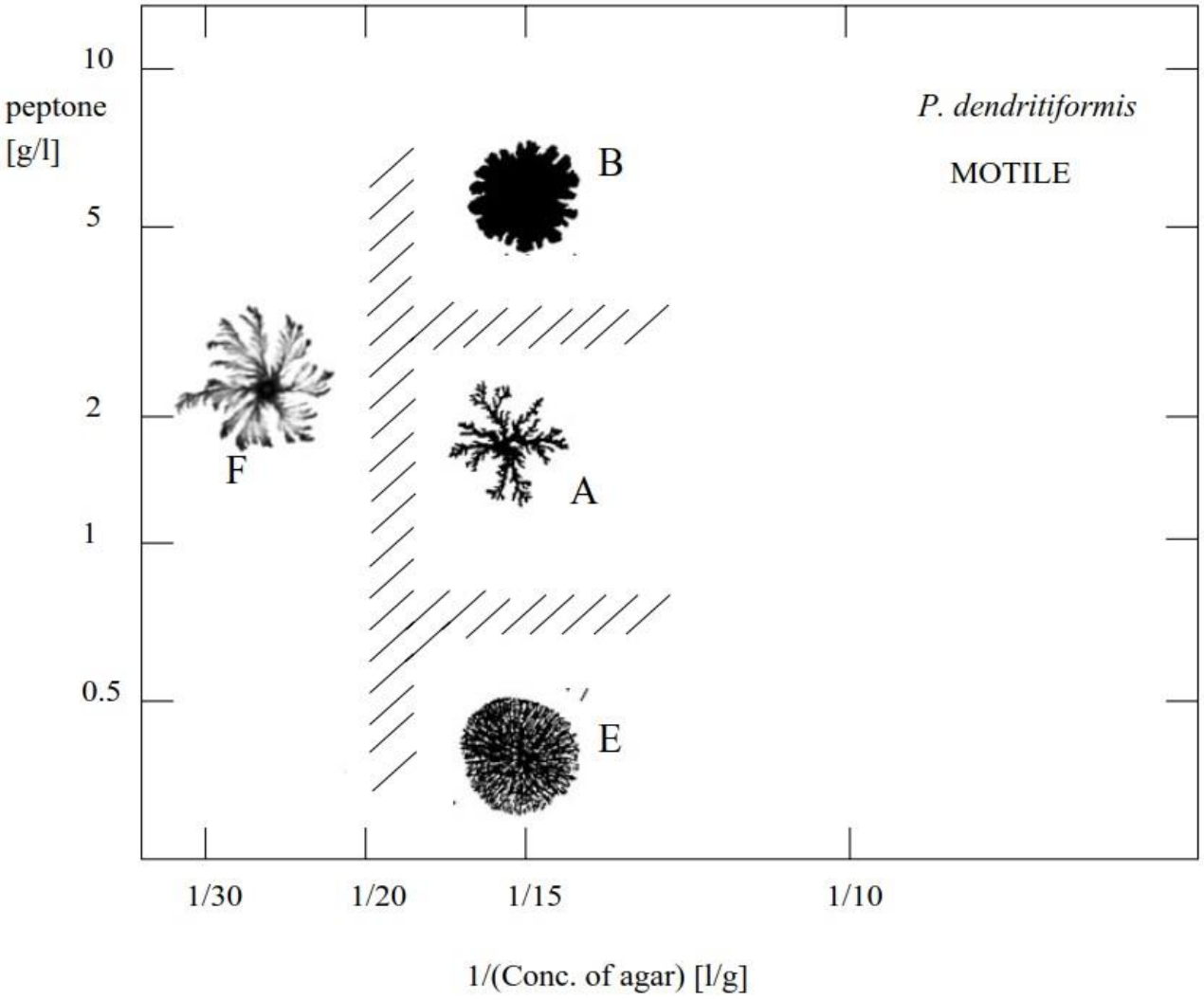


Figure 4.4: Morphology diagram of the strain *Bacillus subtilis* OG-01 (a) and a non motile mutant OG-01b (b) as a function of peptone and agar concentration. The dashed line in (a) indicates the boundary of the active movement of bacterial cells inside the colonies. The morphologies are classified as follows: fractal (A), compact with rough boundary (B), branching with periodic growth phases (C), compact with diffuse boundary (D) and dense branching (E). In the case of the non motile strain the regions A and B seen in (a) expand laterally, while regions C, D and E disappear.

Morphology diagram of *Paenibacillus dendritiformis*



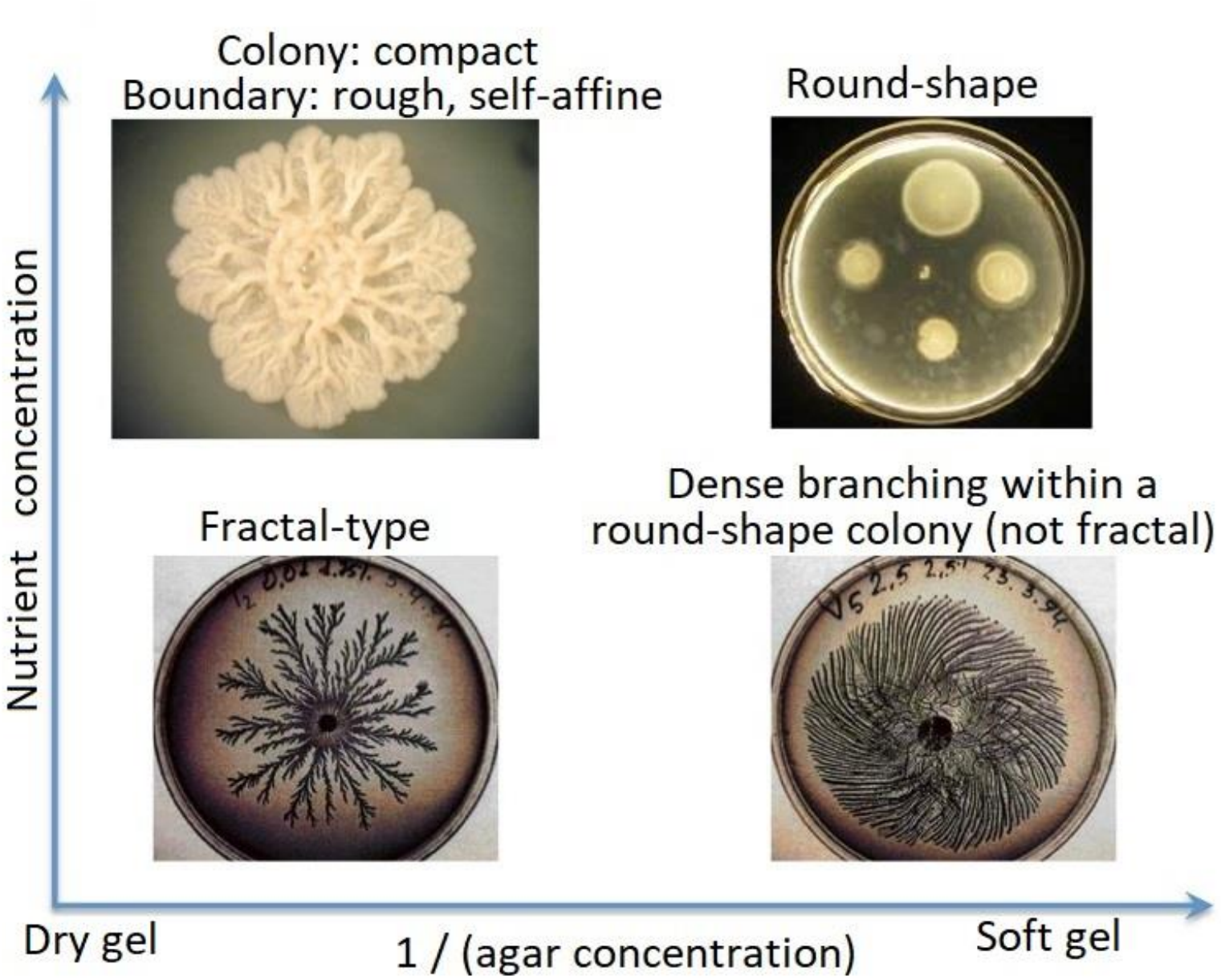
"A": Fractal

"B": Compact with rough boundary

"E": Dense branching

"F": On hard substrate a new, "twisted" morphology appears

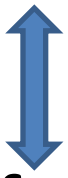
“Summary” of the morphology diagrams



Compact morphology

Abundant nutrient → compact colony

Either smooth or irregular perimeter



Soft gel dry gel

Compact morphology

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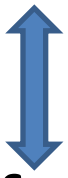


Soft gel → - Bacteria can move

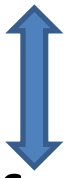
- Takes a few hours to migrate across the dish

- Random walk trajectory

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

– Diffuses due to random translation, and multiplicates

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

Notations:

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

D_ρ : Diffusion coefficient (can be measured as the average displacement of the cells within a given time interval – see later)

∇ : Partial derivative with respect of the space coordinates

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

c : Nutrient concentration

Fisher-Kolmogorov equation – cont.

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

- 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 :

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

(overline: averaging among the cells)

- $f(\rho,c)$: bacterial multiplication
  - 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)
  - We choose cell density units such that $\rho^* = 1$

Fisher-Kolmogorov equation – cont.

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

- We choose $\rho^* = 1$ (threshold-density, above which cell-density can not increase)
- Growth rate decreases as $\rho \rightarrow \rho^* = 1$, and $f(1) = 0$
- The specific form of *f* is unimportant, be will use

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

which satisfies the above criteria

- *R (c)* is a function expressing how the proliferation depends on the nutrient concentration

R(c)= for small c values $R \sim c$

for big *c* values *R* is constant

Numerical solution of the Fisher-Kolmogorov equation in 1 D

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

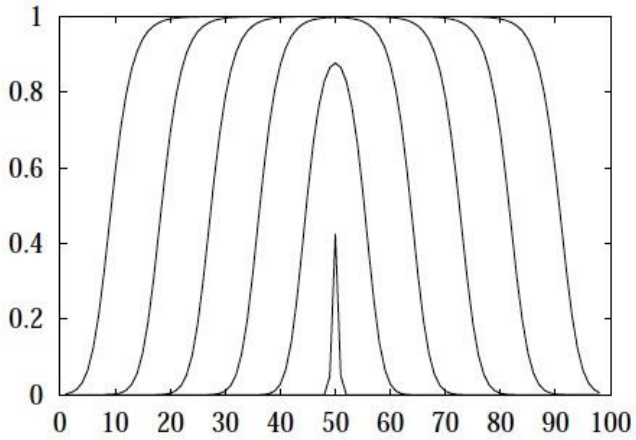


Figure 4.9: Typical result of the numerical integration of the Fisher equation (4.8) starting from a localised perturbation ($D_\varrho = 1$, $f(x) = x - x^2$, i.e., $r = 1$). The $\varrho(x,t)$ curves are plotted for $t = 0, 5, 10, 15, 20$ and 25. The domain grows with a stationary speed of $v = 2$.

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

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

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

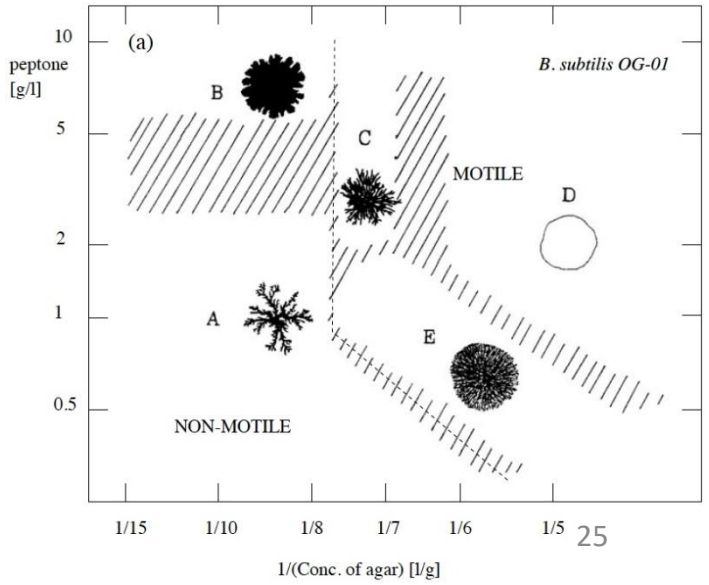
$$\frac{\partial \tilde{\rho}}{\partial t} = D_\rho \tilde{\rho}'' + v\tilde{\rho}' + f(\tilde{\rho})$$

(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.

Velocity selection in experiments

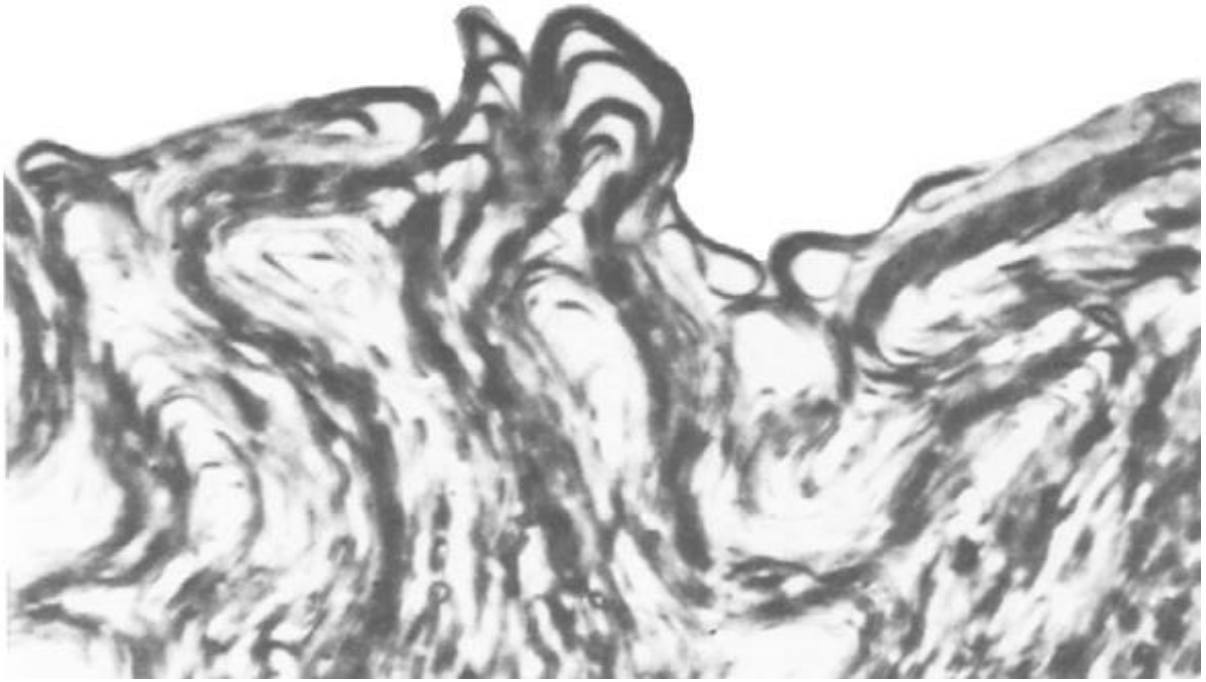
- In reality, there is always one specific growth-speed *v*.
- *Bacillus subtilis* grown in region E
- The spreading of the colony was measured for various nutrient concentration (→ *R(c)~c*)
- $v \sim \sqrt{c}$ held while changing *c* within an order of magnitude



Cell-cell interaction

- When the bacteria are *not* independent during the spreading of the colony (e.g. non-motile cells)
  - Abrupt change in the cell density at the border of the colony
- Propagation of the boundary: expansions of the cell volumes inside the colony
  - The bacteria can not expand to their preferred size, they exert mechanical pressure on their surroundings
  - Large densities: $p \sim \rho - \rho_0$ (ρ_0 threshold density for close-packed colonies)
- For large density values the displacement is: $v = D_0 \nabla(\rho - \rho_0)$
  (D_0: diffusion coef., similarly to D_ρ in the F-K. eq.)
- Modified F-K. eq: $$\partial_t \rho = \begin{cases} D_0 \nabla^2 \rho + f(\rho) & \text{for } \rho > \rho_0 \\ f(\rho) & \text{otherwise} \end{cases}$$
- In such cases the colony boundary is self -affine

The boundary of a *Bacillus subtilis* colony (OG-01 strain) grown on hard agar



Long bundles of chains of individual cells consist the colony of the *B. subtilis* OG-01 strain grown on hard agar. Note the abrupt change in cell density at the boundary.