

Stochastic modeling of the growth of *Escherichia coli*

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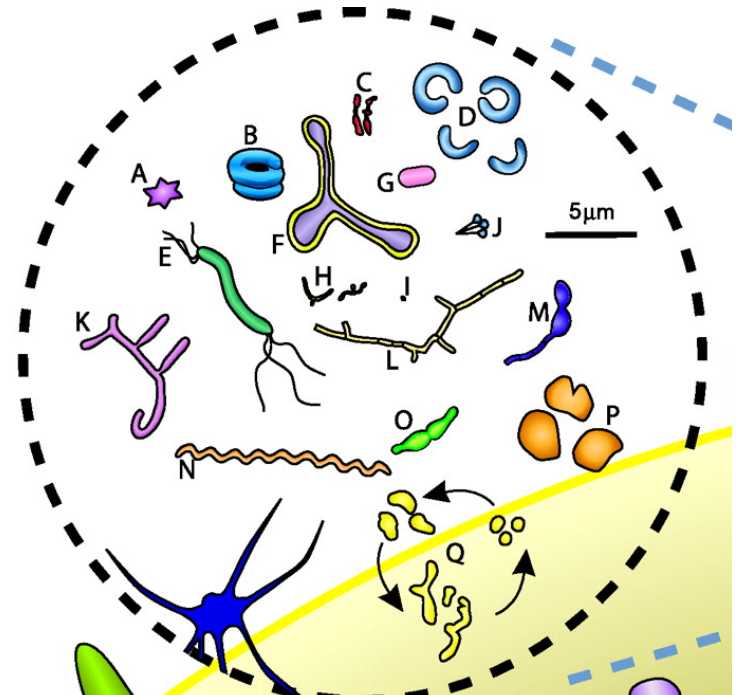
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What are bacteria?

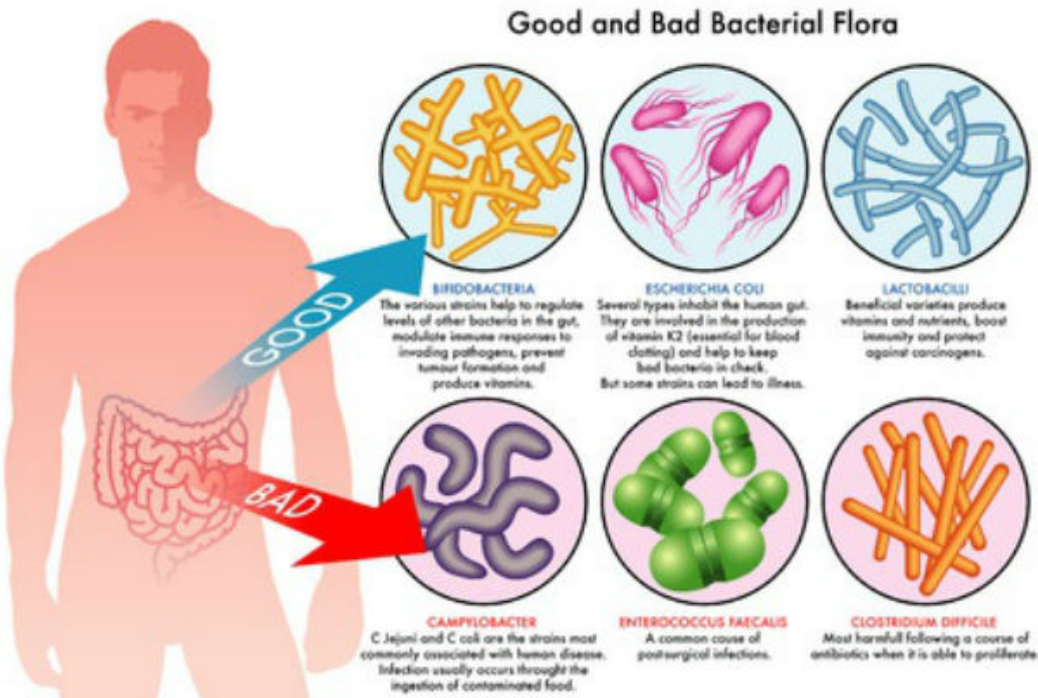
- Tiny unicellular organisms



Kevin D. Young Microbiol. Mol. Biol. Rev. 2006;
doi:10.1128/MMBR.00001-06

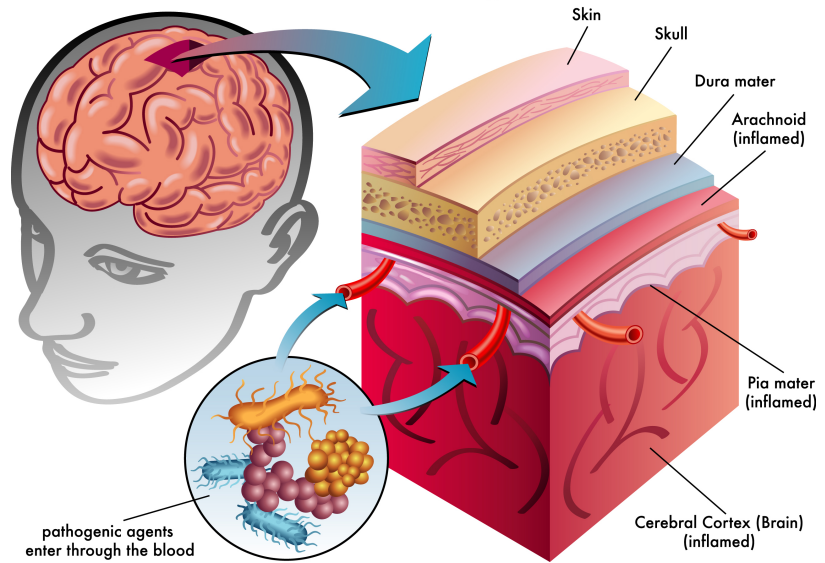
Population size:
Large!

In the human body: 10^{13}

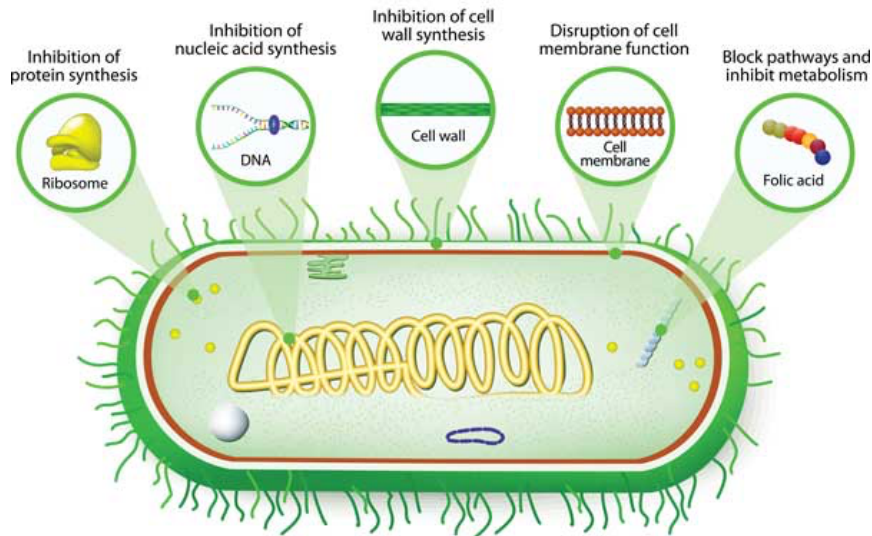


When bacteria grow in our body they can cause infection

Bacterial Meningitis

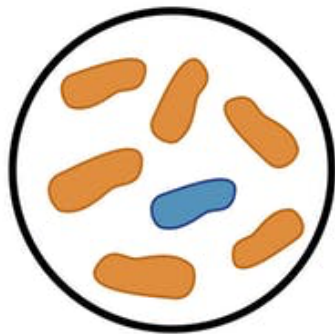


MECHANISMS OF ANTIBIOTIC ACTION

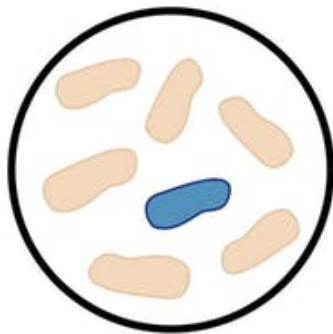


Antibiotics
only kill
growing
bacteria

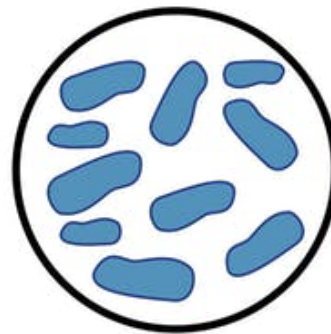
HOW ANTIBIOTIC RESISTANCE HAPPENS



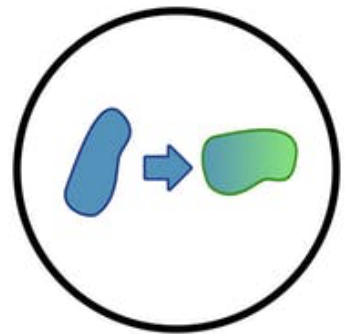
Lots of germs
and some are
drug resistant



Antibiotics kill the bacteria
causing the illness as well as
the good bacteria protecting
the body from infection



The drug resistant
bacteria is now able
to grow and take over



Some bacteria give
their drug resistance to
other bacteria



- Normal bacterium

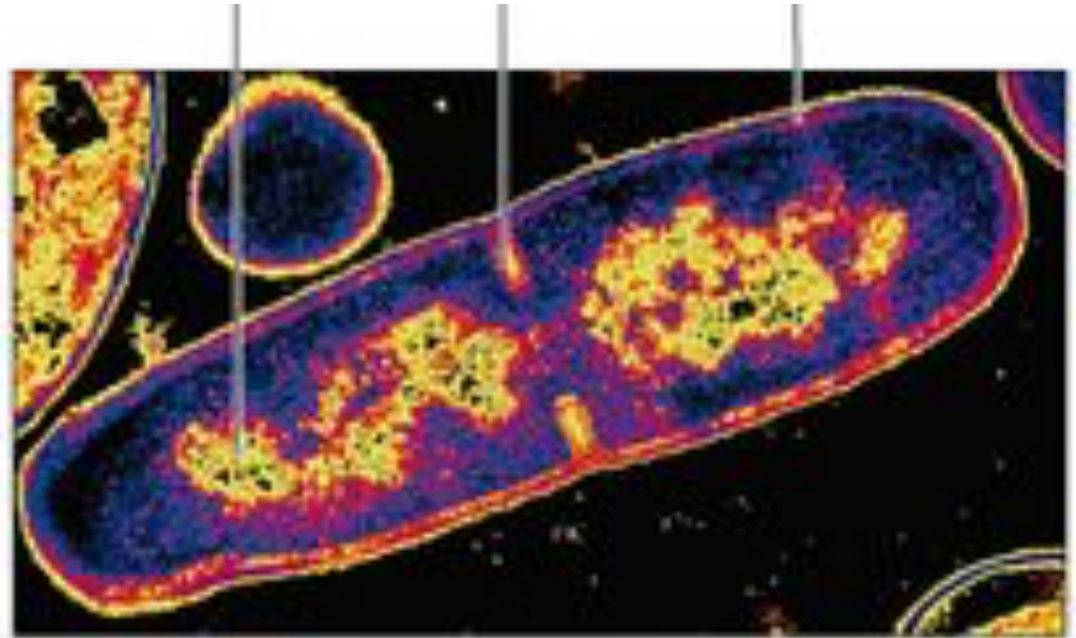
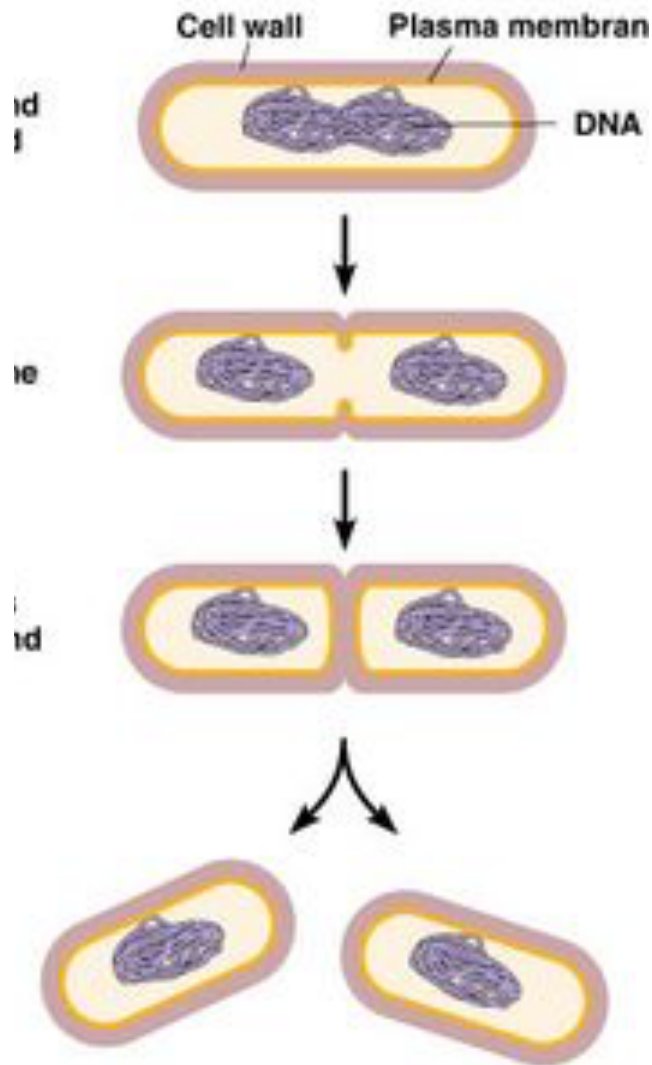


- Resistant bacterium

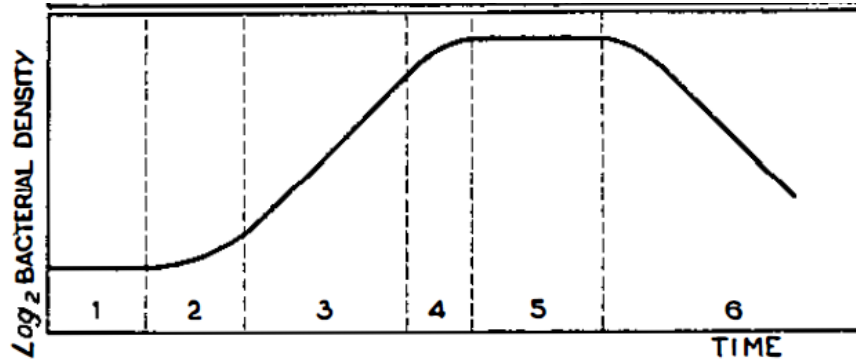


- Dead bacterium

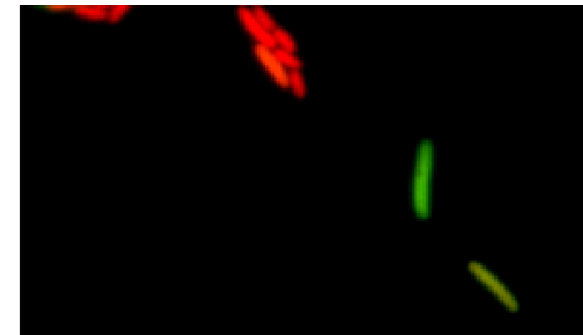
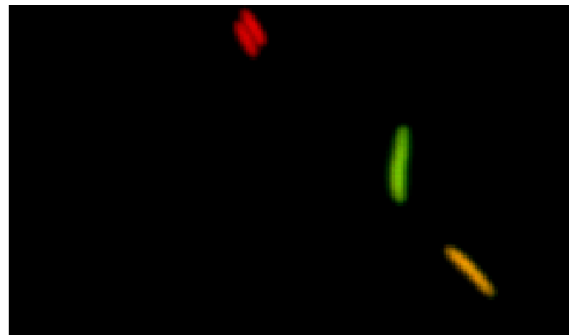
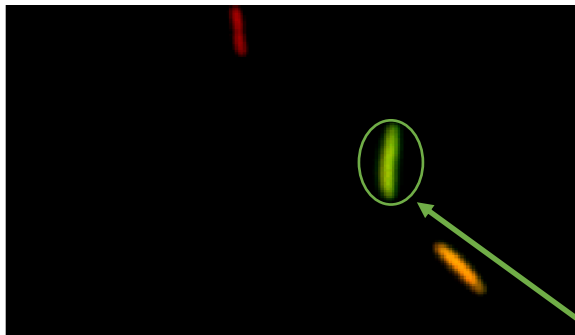
To understand antibiotic resistance we need to understand how bacteria grow



How do bacteria grow? An old question revisited



J. Monod

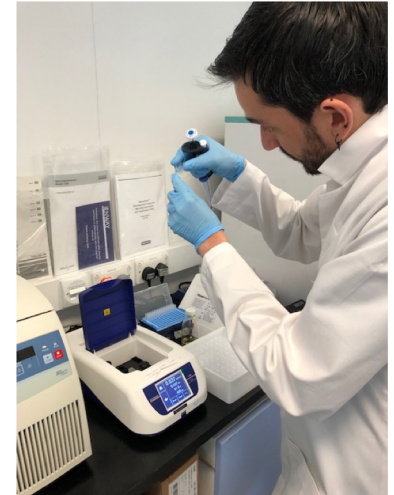
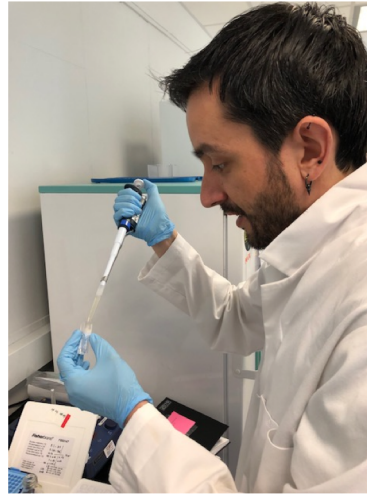


This cell does not grow

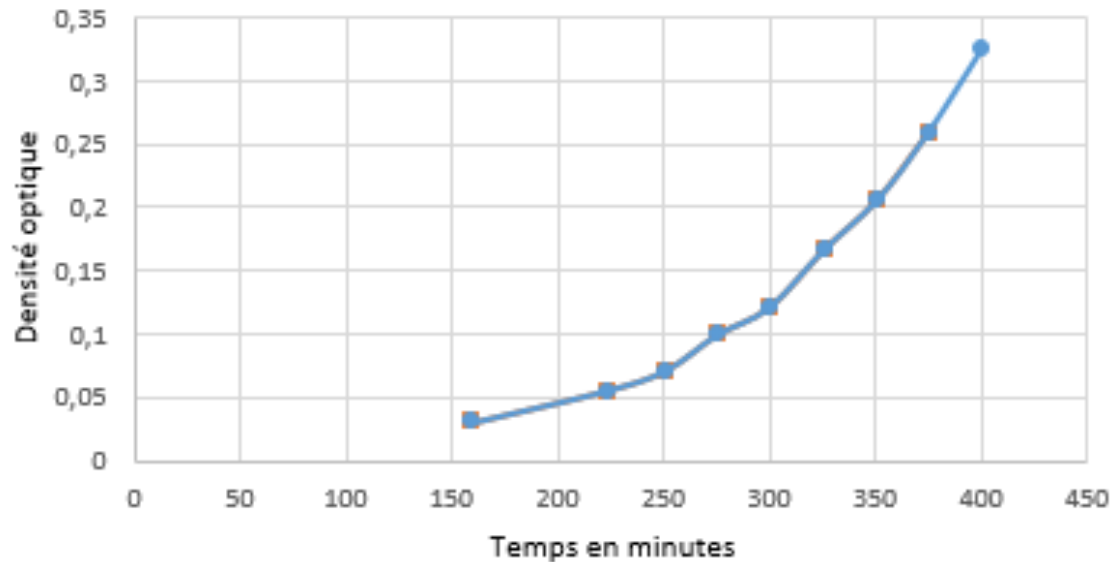
time

J. Monod (1949) Growth of bacterial cultures. *Annual Reviews in Microbiology* 3:371.

Measuring bacterial growth



Actual growth curve (done by Guillaume!)



Observations

- bacteria stop growing when the cell density is high (quiescent)
- They resume growth after a certain (random) amount of time when diluted in fresh growth medium (lag-time)
- Irrespective of cell density some bacteria stop growing for a while: they become persistent (AND antibiotic tolerant)

Can we build a model that predicts the growth curve given the cell to cell variability?

Can we use this model to understand the antibiotics action on the population?

Our aim

- Propose a model that explains the observed curves of population growth.

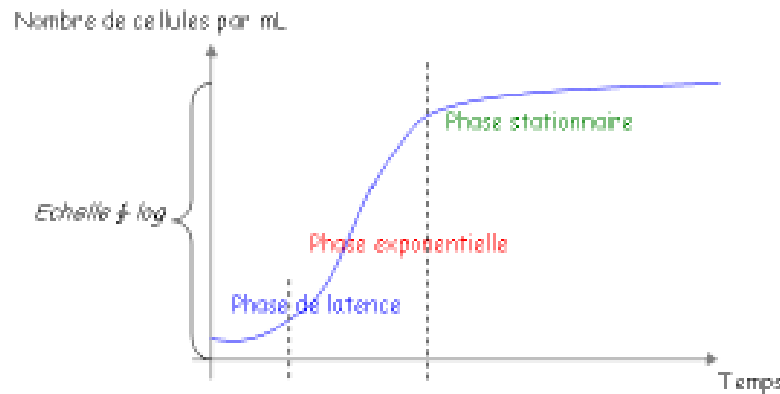


FIGURE 2: Schéma de l'évolution du nombre de bactéries

- Understand the latency phase and the "S" curve without resource constraints.
- Model the quiescence of bacteria.
- Interpret the observations in terms of regulation of growth rates according to the number of bacteria present in the environment.

A model with 3 states based on individuals

Active cells which divide.

Quiescent cells which stop to divide in function of the population state.

Persistent cells which also stop to divide, but at random and during a random time, without interaction with the population.

The population dynamics is described by the Markov process $(\mathcal{N}(t), t \geq 0) \in \mathbb{D}(\mathbb{R}_+, \mathbb{R}_+^3)$, where

$$\mathcal{N}(t) = (N_p(t), N_q(t), N_a(t))$$

gives the number of cells in each state.

We denote by $N(t)$ the total number of cells: $N(t) = N_p(t) + N_q(t) + N_a(t)$.

Meriem's Information:

- The mean of the persistence time is 5 h.
- The cells divide every 30 mn in average when the number of cells is small.
- If the cells number attains some threshold \hat{S} , the birth rate is divided by 2.
- The active cells don't divide anymore if their number attains a certain level.
- An active cell becomes quiescent when there are many cells in the medium.
- A quiescent cell becomes active when there is a few number of cells in the medium.
- At the initial time of each biological experiments, there are approximatively one persistent cell, 10 000 quiescent cells and no active cell.

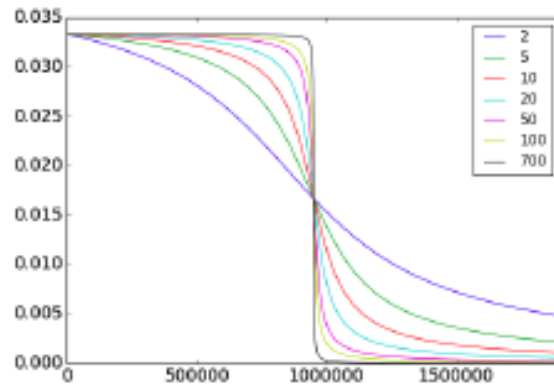
The Transitions

Births:

In a population of size N , the active cells **divide at rate** $\hat{\mu}(N)$ given by

$$\hat{\mu}(N) = b \left(1 - \frac{\arctan(\frac{AN}{\hat{S}} - \gamma) + \arctan(\gamma)}{\frac{\pi}{2} + \arctan(\gamma)} \right),$$

where γ , A and \hat{S} are positive parameters, γ and A being chosen such that $\hat{\mu}(\hat{S}) = b/2$.



Dependence on γ

For *Escherichia coli*, $b = \frac{1}{30}$ in the growing phase.

Deaths: Each active cell **dies at rate** d .

Transfer

- A persistent cell becomes active at rate $r_p : r_p = \frac{1}{300}$.
- An active cell becomes persistent at rate $\tau_p > 0$.
- A quiescent cell becomes active at rate $r_q(N_a) = \frac{\hat{\alpha}}{\hat{\beta} + N_a}$.
- An active cell becomes quiescent at rate $\tau_q N$.

$\tau_p, \tau_q, \hat{\alpha}, \hat{\beta}$ are positive parameters.

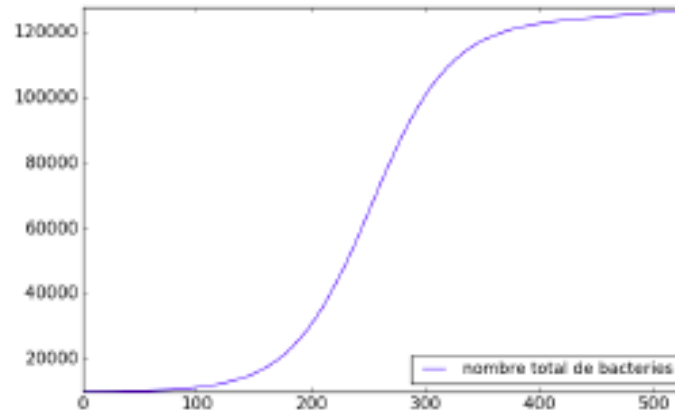
Transitions in the population (N_p, N_q, N_a) , with

$$N = N_p + N_q + N_a.$$

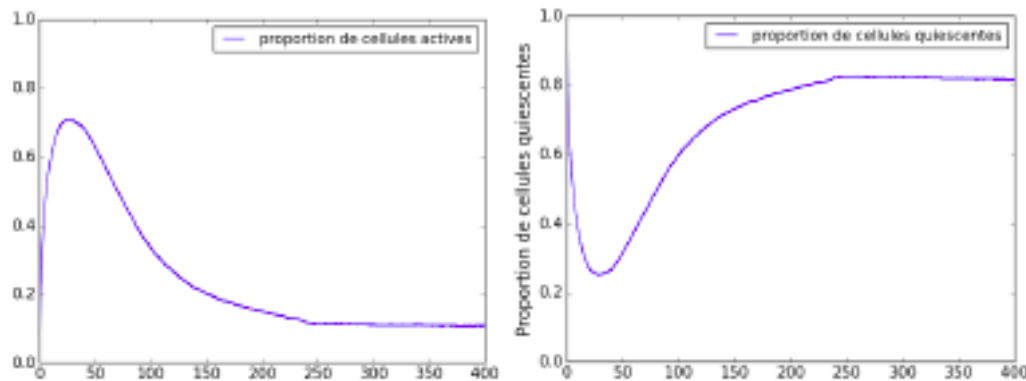
- $(N_p, N_q, N_a) \longrightarrow (N_p - 1, N_q, N_a + 1)$ at rate $r_p N_p$,
- $(N_p, N_q, N_a) \longrightarrow (N_p + 1, N_q, N_a - 1)$ at rate $\tau_p N_a$,
- $(N_p, N_q, N_a) \longrightarrow (N_p, N_q - 1, N_a + 1)$ at rate $r_q(N_a)N_q = \frac{\hat{\alpha}}{\hat{\beta} + N_a} N_q$,
- $(N_p, N_q, N_a) \longrightarrow (N_p, N_q + 1, N_a - 1)$ at rate $\tau_q N N_a$,
- $(N_p, N_q, N_a) \longrightarrow (N_p, N_q, N_a + 1)$ at rate $\hat{\mu}(N) N_a$,
- $(N_p, N_q, N_a) \longrightarrow (N_p, N_q, N_a - 1)$ at rate $d N_a$.

Simulations for $\hat{\alpha} = 0.5$, $\hat{\beta} = 2500$, $\tau_p = 10^{-4}$, $\tau_q = 5 \times 10^{-7}$, $\hat{S} = 150000$, $\gamma = 200$, $K = 10^4$.

Total number of cells

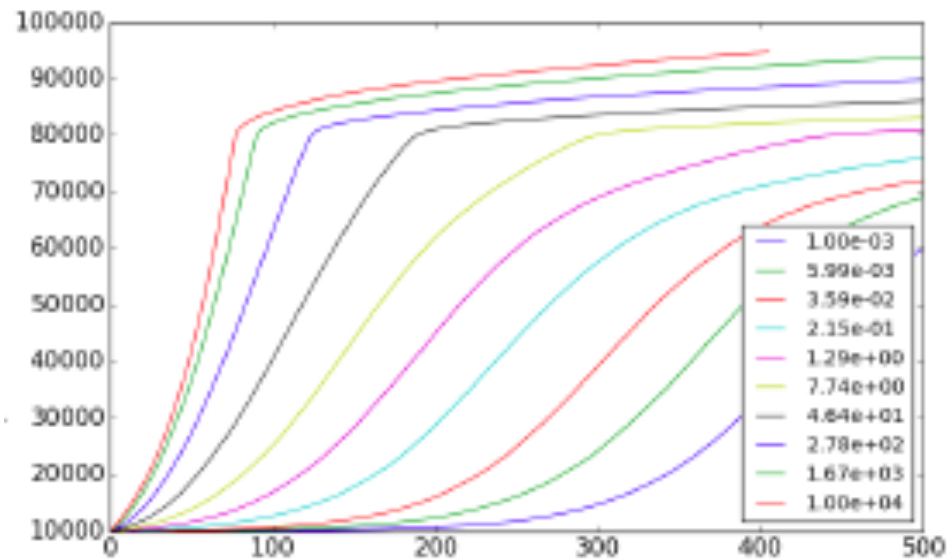


Proportion of active cells and quiescent cells.



Influence of $\hat{\alpha}$ on the lag

Total number of cells in function of $\hat{\alpha}$: smaller is $\hat{\alpha}$, longer is the lag.



Large population

Initial condition: $\mathcal{N}(0) = (1, 10^4 - 1, 0)$.

We have a size scale of 10^4 : large population parameter.

We introduce a scale parameter K .

The general model: initial condition $(1, K, 0)$.

We look for an approximation for large K .

Let us set

$$X^K = \frac{\mathcal{N}^K}{K}$$

with $\mathcal{N}^K(0) = (1, K, 0)$

\hat{S} replaced by $S K \implies \hat{\mu}$ replaced by μ .

$\hat{\alpha}$ and $\hat{\beta}$ replaced by αK and βK .

The limiting system

Proposition: (Kurtz 1971)

Let us fix $T > 0$. When $K \rightarrow \infty$, the process $(X_t^K)_{t \leq T}$ converges in probability to the solution $(y_t)_{t \leq T}$ of the system:

$$\frac{dy_1}{dt} = \tau_p y_3 - r_p y_1;$$

$$\frac{dy_2}{dt} = \tau_q (y_1 + y_2 + y_3) y_3 - \frac{\alpha}{\beta + y_3} y_2;$$

$$\frac{dy_3}{dt} = r_p y_1 + \frac{\alpha}{\beta + y_3} y_2 + \mu (y_1 + y_2 + y_3) y_3 - (\tau_p + \tau_q (y_1 + y_2 + y_3) + d) y_3.$$

avec $y(0) = (0, 1, 0)$.

Proof: argument of compactness-identification-uniqueness.

Study of the system

There is existence and uniqueness of the positive solution. In addition, the solution is bounded and stays in the compact set $[0, \mu^{-1}(d)]^3$.

There are two equilibria.

$(0, 0, 0)$ is an unstable equilibrium.

The second equilibrium (repartition of the population in the stationary phase), is

$$y^{eq} = \left(\frac{\tau_p}{r_p} y_3^{eq}, \mu^{-1}(d) - \left(\frac{\tau_p}{r_p} + 1 \right) y_3^{eq}, y_3^{eq} \right),$$

with

$$y_3^{eq} = \frac{\alpha \sqrt{\left(4\mu^{-1}(d) \frac{\tau_q}{\alpha} + \left(\frac{\tau_q \beta}{\alpha} + 1 + \frac{\tau_p}{r_p} \right)^2 \right)} - \alpha - \tau_q \beta - \frac{\tau_p}{r_p} \alpha}{2\tau_q}.$$

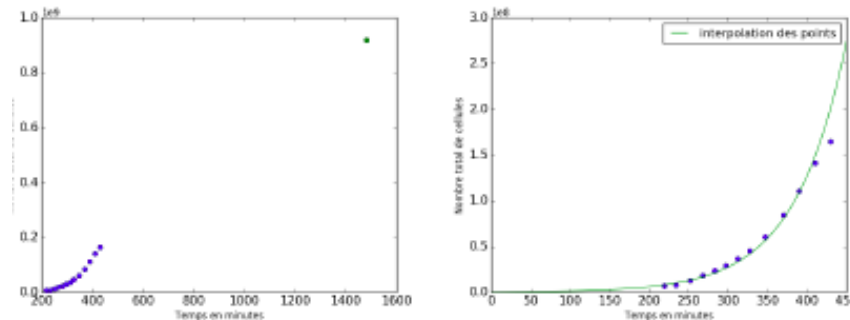
It's a stable equilibrium, globally attractive.

Remark: the total size at equilibrium can be computed:

$$N^* = y_1^{eq} + y_2^{eq} + y_3^{eq} = \mu^{-1}(d).$$

Calibration of the model

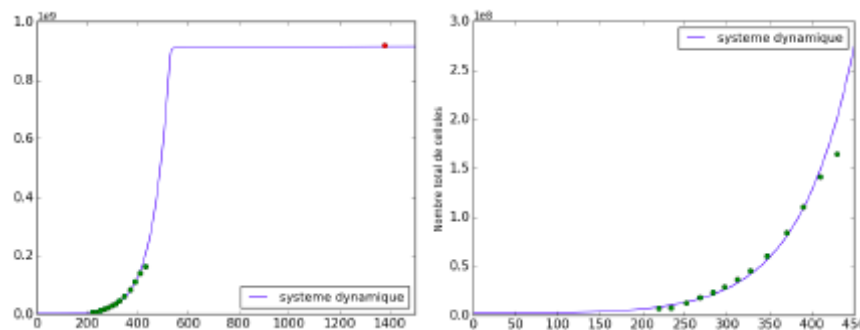
First experiment: medium with glucose and amino-acids. The temperature was cold and the division took a longer time than waited. ($> 30mn$).



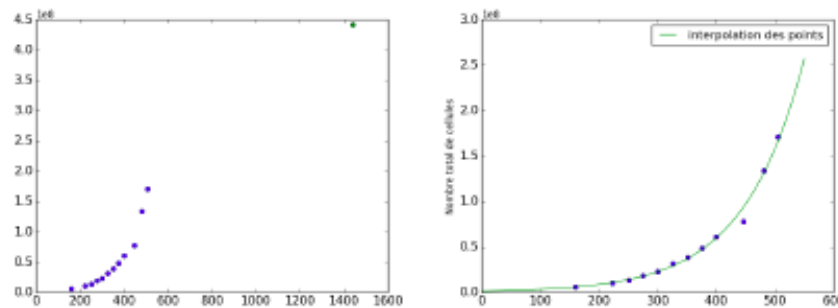
Stochastic calibration method with an algorithm of mutation-selection.

We take $K = 2 \times 10^6$, $\gamma = 90$, $d = \mu(N/K)$.

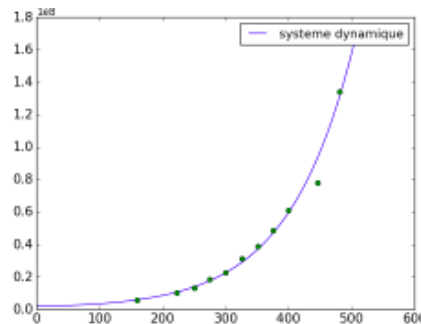
We calibrate 6 parameters: $(\alpha, \beta, \tau_p, \tau_q, S, b) \in [0, 1]^4 \times [100, 1000] \times [0, 1]$.



Second experiment: in a medium with glucose but without amino-acids: the cells have to produce their amino acids and then, the division time is longer.



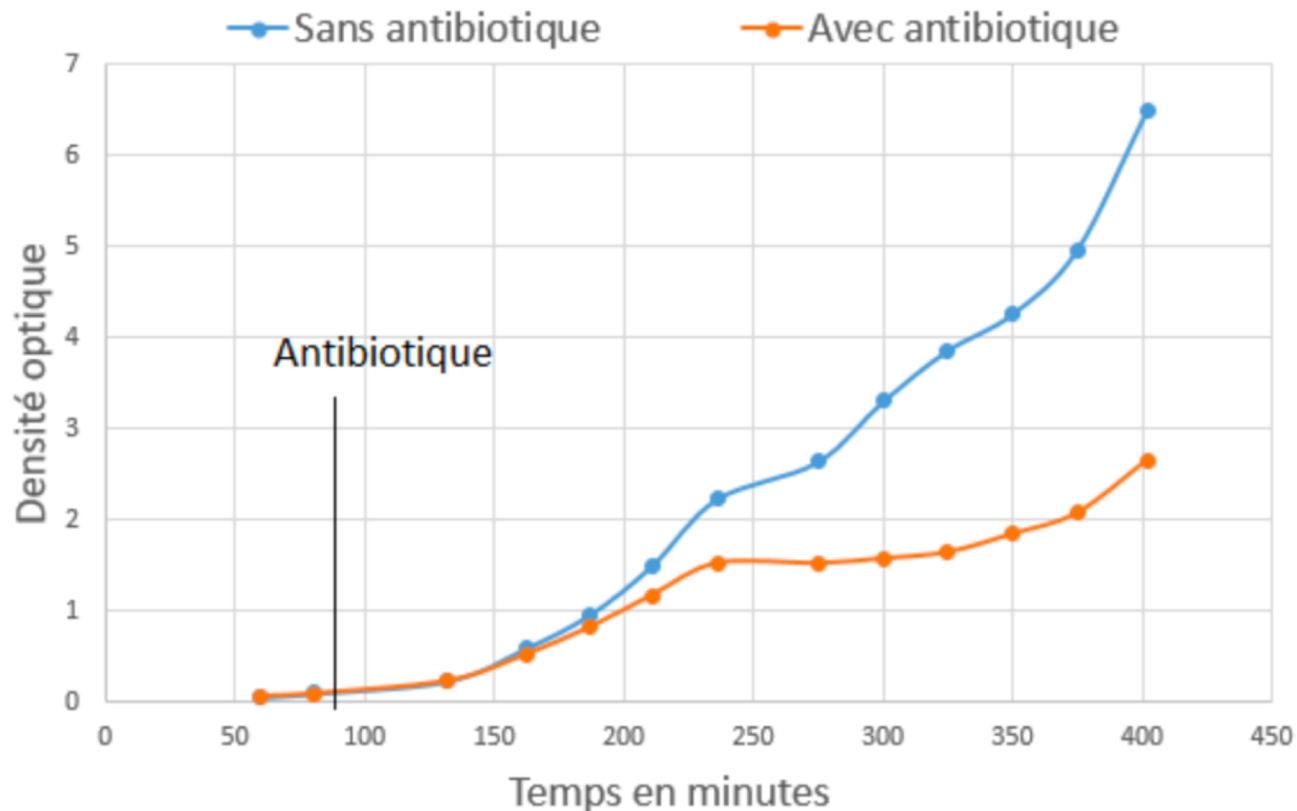
After calibration:



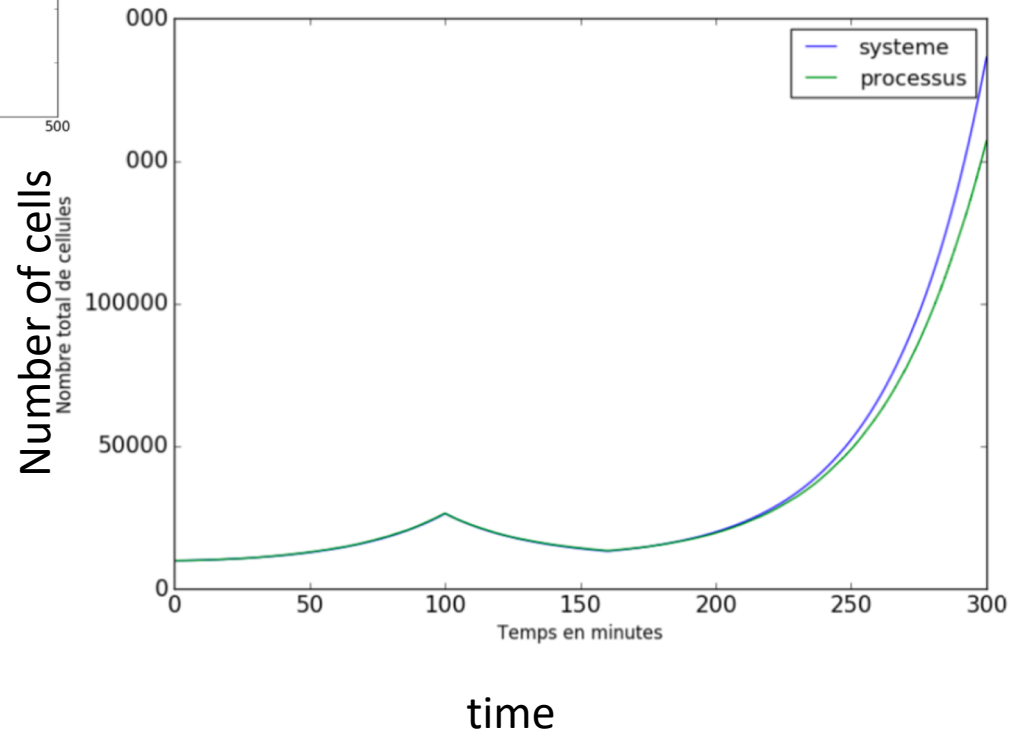
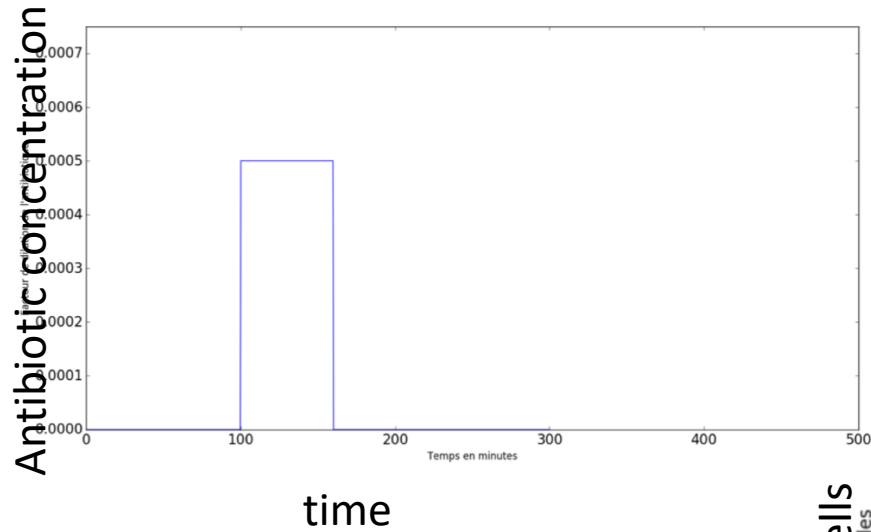
We note that τ_q is larger and b is smaller (which gives a longer division time) than in the previous experiment.

Conclusion so far

- We have model that can explain the growth curve taking into account single cell behavior
- What happens if there are antibiotics?



Initial simulations recapitulate (*qualitatively*) system behaviour



Take home messages

- To understand antibiotics action we need quantitative single cell based models of bacterial growth
- Our model recapitulates the main features of the “growth curve”
- Antibiotic model : work in progress....