Mechanism-based pharmacodynamic modelling of bacterial growth inhibition by antibiotics

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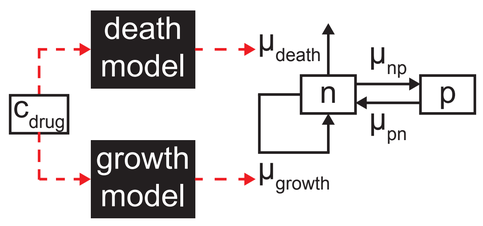
Objective

Typical drug-effect models directly link exposure to antibiotics to bacterial population growth. They rarely account for known mechanisms of action of the drug — which are particularly relevant for the analysis of synergistic or antagonistic effects of drug combinations. Our aim was to develop a generic pharmacodynamic model which allows for mechanistic integration of antimicrobial drug effects on the cellular level to predict the impact on bacterial growth.

Methods



- Two sub-populations: Normal (n) and persisting cells (p), which switch their phenotype with rate constants μ_{np} and μ_{pn}
- Antibiotic concentration c_{drug} acts on some **death model** and some **growth model**
- Transition constant λ is predicted by some **transition model**
- Growth is limited by carrying capacity $N_{\max}$

$$\begin{aligned}\mathrm{d}n/\mathrm{d}t &= \mu_{\mathrm{growth}}(1-(n+p)/N_{\max})n - \mu_{\mathrm{death}}n - \mu_{\mathrm{np}}n + \mu_{\mathrm{pn}}p\\ \mathrm{d}p/\mathrm{d}t &= \mu_{\mathrm{np}}n - \mu_{\mathrm{pn}}p\\ \mathrm{d}\mu_{\mathrm{growth}}/\mathrm{d}t &= \lambda(\mu_{\mathrm{growth,adapted}} - \mu_{\mathrm{growth}})\end{aligned}$$

Death model

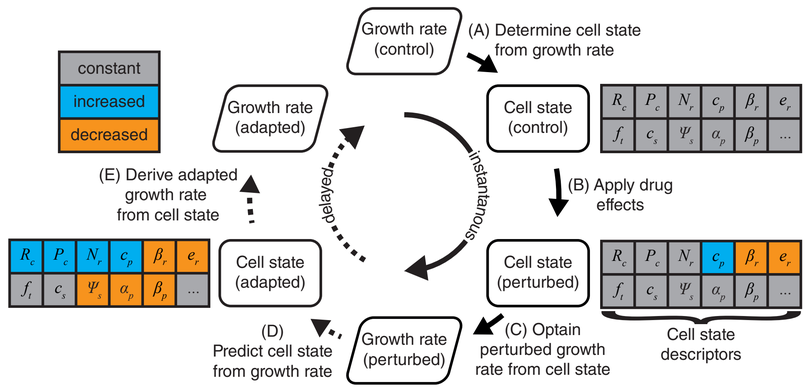
Based on data in [11], we assumed, that the baseline probability for death per cell is constant for i) all generations (no senescence) and ii) all phases of the cell cycle with $K_{\mathrm{death}} = 0.01$.

$$\mu_{\mathrm{death}} = K_{\mathrm{death}} \cdot \mu_{\mathrm{growth,control}} + \frac{E_{\max,\mathrm{death}} \cdot c_{\mathrm{drug}}^{\gamma_{\mathrm{death}}}}{EC_{50,\mathrm{death}}^{\gamma_{\mathrm{death}}} + c_{\mathrm{drug}}^{\gamma_{\mathrm{death}}}}$$

Growth model

The mechanism of action is exemplified for **protein biosynthesis inhibitors** tetracycline and chloramphenicol, which inhibit translation during the elongation phase. They share the kinetic properties of long binding times to their ribosomal targets (several minutes) — compared to the typical time a ribosome spends in the elongation phase ($\approx 280 \cdot 0.04$ s for *E. coli*).

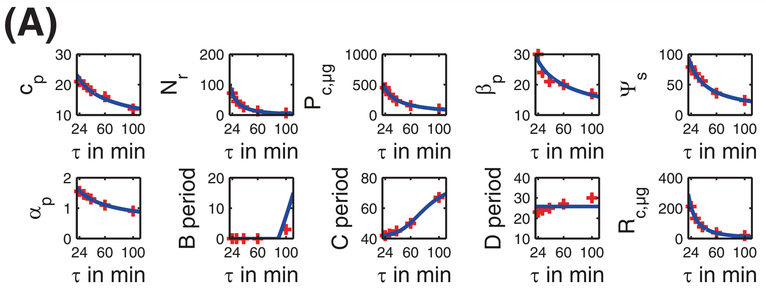
The growth model included cell states and corresponding growth rate constants. A delay for adaptational processes was predicted by a **transition model**. The partitioning of the cell state is in accordance with a RelA and SpoT homologue regulation network (relaxed response).



Parameter abbreviations and units: β_{r} fraction of active ribosomes; c_{p} peptide chain elongation rate per active ribosome in aa/s; e_{r} ribosomal efficiency in aa/s ($= c_{\mathrm{p}}\beta_{\mathrm{r}}$); N_{r} number of 10^3 ribosomes per cell; $P_{\mathrm{c},\mu\mathrm{g}}$ protein mass per 10^9 cells in µg; $R_{\mathrm{c},\mu\mathrm{g}}$ RNA mass per 10^9 cells in µg; β_{p} fraction of active RNA polymerase in %; Ψ_{s} fraction of active RNA polymerase synthesizing rRNA and tRNA in %; α_{p} fraction of total protein that is RNA polymerase in % ; M_{c} cell dry weight per 10^9 cells in µg; $\mathrm{nucl./prib.}$ ribonucleotide residues per rRNA precursor; $\mathrm{aa/pol.}$ amino acid residues per RNA polymerase core; f_{t} fraction of stable RNA that is tRNA; c_{s} stable RNA chain elongation in nucl/s; $\mathrm{nucl./rib.}$ ribonucleotide residues per 70S ribosome; f_{s} fraction of RNA that is stable RNA.

(A)

A functional link between doubling time $\tau = 60\log(2)/\mu_{\mathrm{growth}}$ and cell state descriptors (blue line) was estimated based on data in [1] (red crosses) and identity condition $\mu = f(\mathrm{CellState}(\mu))$ with μ defined in step (E).



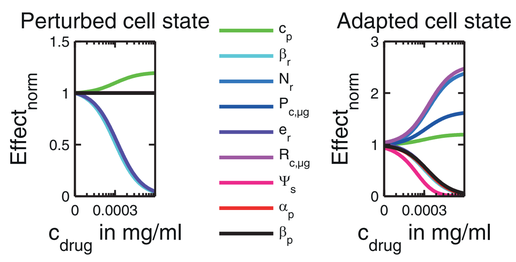
(B)

$$\beta_{\mathrm{r,perturbed}} = \beta_{\mathrm{r,control}} \cdot \left(1 - \frac{c_{\mathrm{drug}}^{\gamma_{\mathrm{growth}}}}{\mathrm{EC}_{50,\mathrm{growth}}^{\gamma_{\mathrm{growth}}} + c_{\mathrm{drug}}^{\gamma_{\mathrm{growth}}}}\right) \qquad c_{\mathrm{p,perturbed}} = c_{\mathrm{p,control}} + \frac{(c_{\mathrm{p,max}} - c_{\mathrm{p,control}}) \cdot c_{\mathrm{drug}}^{\gamma_{\mathrm{growth}}}}{\mathrm{EC}_{50,\mathrm{growth}}^{\gamma_{\mathrm{growth}}} + c_{\mathrm{drug}}^{\gamma_{\mathrm{growth}}}}$$

We applied Liebig's law of the minimum by deriving the inverse of the most limiting cell state descriptor in $\mathrm{CellState}_{\mathrm{perturbed}}$ — for chloramphenicol and tetracycline this is the ribosomal efficiency ($e_{\mathrm{r}} = \beta_{\mathrm{r}} \cdot c_{\mathrm{p}}$)

(C) $\mu_{\mathrm{growth,perturbed}} = 60\log(2)/f_{e_{\mathrm{r}}}^{-1}(e_{\mathrm{r,perturbed}})$

In the perturbed cell state, the fraction of active ribosomes β_{r} was modulated by c_{drug} with an Emax model. The remaining active ribosomes could benefit from increased amino acid pool levels and thus resulting higher peptide chain elongation rate c_{p}, limited by $c_{\mathrm{p,max}}$.



(D)

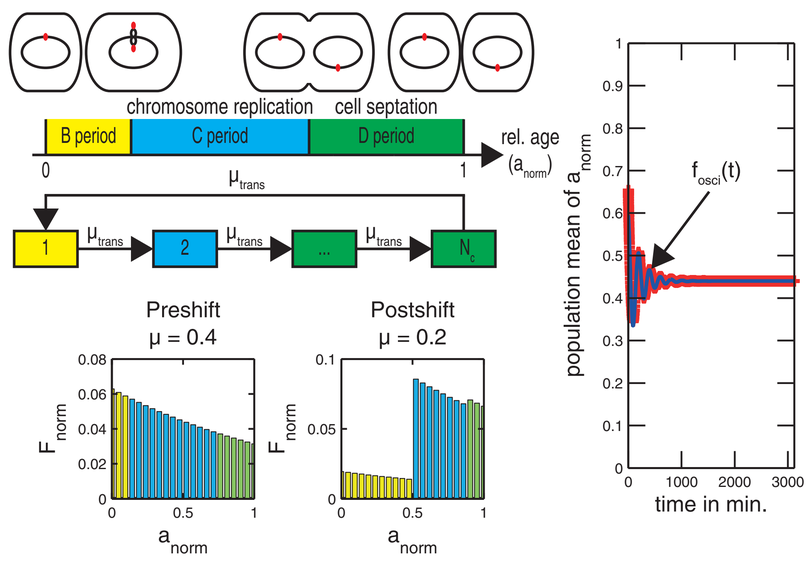
In the adapted cell state, RNA related parameters exhibited a relaxed response linked to $c_{\mathrm{p,perturbed}}$. RNA polymerase related parameters followed a stringent response linked to $\mu_{\mathrm{growth,perturbed}}$. Parameters directly lowered by drug action remained on the perturbed level.

(E) $\mu_{\mathrm{growth,adapted}} = \dfrac{60}{[(\mathrm{nucl./prib.})(\mathrm{aa/pol.})/(1-f_{\mathrm{t}})]^{0.5}}(\Psi_s\alpha_p\beta_p\beta_r c_s c_p)^{0.5}$

Transition model

The aim is to predict the time needed for transit between pre-shift and post-shift growth rate.

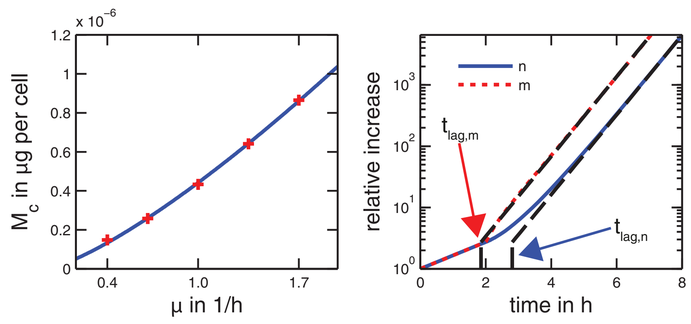
- a_{norm} describes the gradual progress of a single cell in the cell cycle and was ideally distributed with $F = 2^{1-a_{\mathrm{norm}}}$
- A re-scaled and shifted post-shift distribution gave initial values for a transit compartment cell cycle model
- N_{c} and μ_{trans} was given by the known variance of the resulting Erlang distribution ($\sigma^2 = N_{\mathrm{c}}/\mu_{\mathrm{trans}}^2$)
- Decay rate of the envelope of $f_{\mathrm{osci}}(t)$ gave transition rate constant λ



Results

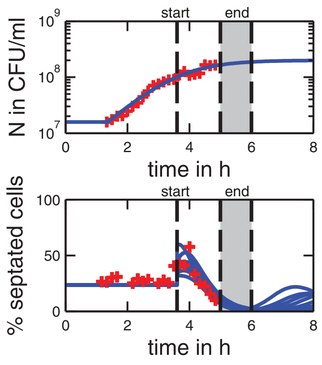
Predictions of lag between increase in cell number and population mass

In this experiment additional nutrients were added into the growth medium at $t = 2$ h. A shift-up lead to increasing mass per typical cell M_{c}. The transition model predicted different lag times for mass increase ($m = nM_{\mathrm{c}}(\mu)$) and increase in cell number (n).



For *E. coli* B/r, with parameters estimated from data in [1] (red crosses), we can quantitatively predict $\Delta t_{\mathrm{lag}} \approx 1$ h.

Prediction of septation dynamics of *B. subtilis*



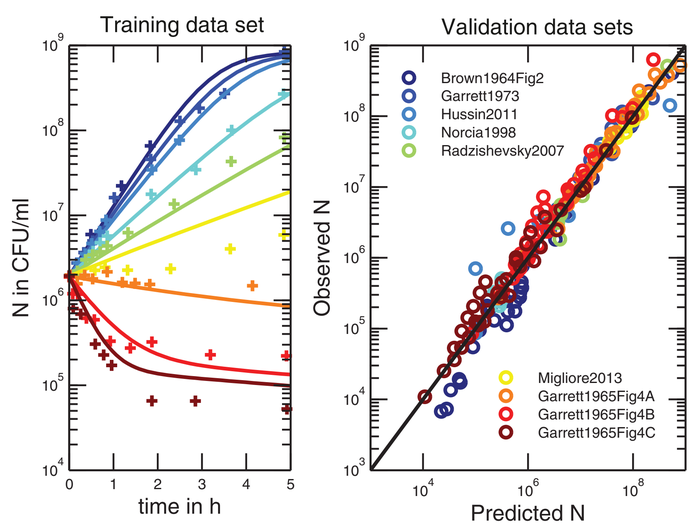
Population dynamics (N) were fitted by a logistic growth function with lag time. Its derivative gave pre- and post-shift growth rates.

- Experimental data as red crosses from [3]
- Predictions from the transition model for 10 post-shift growth rates as blue lines

Oscillations in age distribution faded out ≈ 4 h after start of transition.

Prediction of population dynamics for *E. coli* exposed to tetracycline

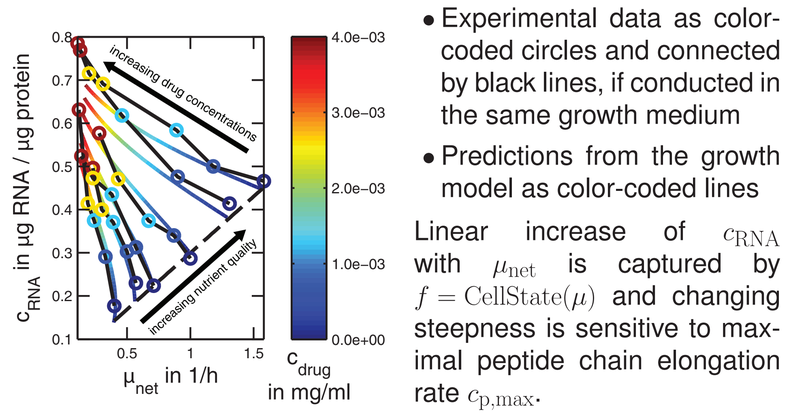
Two drug specific EC_{50} parameters were estimated in a training data set for *E. coli* B/r and static concentrations up to 8 µg mL^{-1} of tetracycline (color coded in training data set from [2].



For validation purposes, data from different strains (B/r, ATCC 25922, ATCC 51A0150 and MG165) and growth media (Antibiotic medium 3 and Mueller Hinton) were used [2, 5, 6, 8, 9, 7, 4]. Drug specific parameters were scaled accordingly for each strain. We see very good agreement in these data sets.

Predictions of intracellular RNA concentration for *E. coli* exposed to chloramphenicol

RNA concentrations (c_{RNA}) during exponential growth were measured in several growth media and resulting growth rates μ_{net} [10]. Chloramphenicol was added in various concentrations.



- Experimental data as color-coded circles and connected by black lines, if conducted in the same growth medium
- Predictions from the growth model as color-coded lines

Linear increase of c_{RNA} with μ_{net} is captured by $f = \mathrm{CellState}(\mu)$ and changing steepness is sensitive to maximal peptide chain elongation rate $c_{\mathrm{p,max}}$.

Summary

Exploitation of cell state — growth rate interrelation enables **flexible integration of antibiotic drug effects**. **Separation of system and drug specific parameters** allows transfer of information between experiments. **Biological interpretation** of parameters can guide follow-up experiments and give insight into cellular responses to exposure to antibiotics.

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