

Modeling of antibiotic concentration reduction in periplasmic space

1 Steady state description

Let a and a_p be the antibiotic concentration outside the cell and inside the periplasmic space respectively. Let E be the enzyme (β -lactamase) concentration in the periplasmic space. The ODE for the a_p is

$$\frac{da_p}{dt} = \sigma(a - a_p) - \frac{k_{cat}}{k_M} a_p E, \quad (1)$$

where σ is the flux rate constant for the antibiotic across the cell surface, and k_{cat} and k_M are standard enzyme-kinetic parameters. The first term on the right hand side corresponds to antibiotic flux into the periplasmic space, whereas the second term corresponds to breakdown of antibiotic in the cell by enzymatic activity. Assuming the extracellular antibiotic concentration a to be constant, the full solution to the above equation is

$$a_p(t) = a_{p,steady} + [a_{p,steady} - a_{p,initial}]e^{-\frac{t}{\tau}}$$

where

$$\begin{aligned} a_{p,steady} &= \frac{a}{R}, \\ \tau &= \frac{1}{\sigma R}, \end{aligned} \quad (2)$$

and

$$R = 1 + \frac{E k_{cat}}{\sigma k_M}. \quad (3)$$

Thus the steady-state concentration $a_{p,steady}$ in the periplasmic space is lower than the extracellular concentration by a factor of R , and the steady state value is attained over a time-scale τ as given above. (Note that the intracellular concentration must eventually deviate from the value $a_{p,steady}$ as the concentration a starts decreasing due to antibiotic breakdown.)

There is a simple relationship between R and MIC. Denote the wild-type MIC as MIC_{WT} . Note that the wild-type has no β -lactamase, and therefore it has $a_{p,steady} = a$ (at least approximately); this follows from (2) and the fact that $R = 1$ when $E = 0$. Thus, for any strain, $a_{p,steady} = MIC_{WT}$ can be taken as the minimum *periplasmic* concentration at which the cells do not grow. The mutants with the enzyme increase their MIC by reducing the periplasmic concentration $a_{p,steady}$ to below the extracellular concentration a . The MIC of a strain can therefore be found by computing the extracellular concentration a at which $a_{p,steady} = MIC_{WT}$. Thus, from (2),

$$R = \frac{MIC}{MIC_{WT}}. \quad (4)$$

Using this approach, the reduced concentration can be computed directly from the MIC, without knowledge of σ , k_{cat}/k_M or E . The values of the reduction factor R and steady periplasmic concentrations for the eight mutants are given in the Excel file. In addition, the values of k_{cat}/k_M from [1] are also noted in the file. We have also used the estimated R and σ to infer values of E (although they have not been used further).

2 Dynamical model with cell growth and antibiotic degradation

The equations used here are

$$\begin{aligned} \frac{dN}{dt} &= \alpha N \left(1 - \frac{N}{N_c}\right) \\ \frac{da_p}{dt} &= -V_{max} a_p + \sigma(a - a_p) \\ \frac{da}{dt} &= -\eta \sigma N(a - a_p) \end{aligned} \quad (5)$$

$$(6)$$

where N is the bacterial population, N_C is the carrying capacity, α is the growth rate, $V_{max} = \frac{k_{cat}}{k_M} \times E$, and η is the volume ratio of the periplasmic space to the medium volume. The first equation models the logistic growth of the population which is assumed to be unaffected by the antibiotic concentration. In the second equation, the first term represents the degradation of periplasmic antibiotic due to enzymatic activity, while the second represents the influx of antibiotic into the periplasmic space. The third equation models change of antibiotic concentration in the medium due to uptake and degradation by the cells.

From the literature [2, 3], we estimate $\sigma \simeq 1.7 \text{ s}^{-1}$. Further, we have used a 30 minutes doubling time for α . The factor η is estimated to be 0.3×10^{-12} [4]. To determine V_{max} , we determined $\frac{k_{cat}}{k_M} \times E$ using (3) where the values of R were estimated empirically through (4).

References

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