

Senescence Can Explain Microbial Persistence

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Summary. It has been known for many years that small fractions of persister cells resist killing in many bacterial colony-antimicrobial confrontations. These persisters are not believed to be mutants. Rather it has been hypothesized that they are phenotypic variants. Current models allow cells to switch in and out of the persister phenotype. Here a different explanation is suggested, namely senescence, for persistence. Using a mathematical model including age structure, it is shown that senescence provides a natural explanation for persistence-related phenomena including the observations that persister fraction depends on growth phase in batch culture and dilution rate in continuous culture.

1 Introduction

It has been observed (Balaban et al., 2004; Bigger, 1944; Gilbert et al., 1990; Greenwood & O’Grady, 1970; Keren et al., 2004B; McDermott, 1958; Moyed & Bertrand, 1983; Sufya et al., 2003; Harrison et al., 2005; Wiuff et al., 2005), dating to Bigger (1944), that many antimicrobials while effective in reducing bacterial populations are unable to eliminate them entirely, even with prolonged exposure. The surviving cells, called persisters, may be small in number – Bigger (1944) reported less than 100 persisters out of $2.5 \cdot 10^7$ cells of *Staphylococcus pyogenes* after exposure to penicillin in some cases for example – but nevertheless are subsequently able upon removal of the challenging agent to repopulate. See Lewis (2001) for a general discussion. This phenomenon has recently gained increased attention in the context of biofilms (Spoering & Lewis, 2001) where the persisting cells have the added protection of a polymeric matrix, making them particularly dangerous (see, e.g., models of Roberts & Stewart, 2004, 2005, Ayati & Klapper, 2007). The protection of microbial populations by persistence, whether formed by senescence or some other mechanism, is expected to be enhanced in biofilms because of the propensity of biofilms to harbor slow-growing or non-growing cells (Drury et al., 1993; Heijnen et al., 1994, Okabe et al., 1997).

Persisters have a number of interesting characteristics:

- Upon reculturing, persister cells enable repopulation. See, for example, Balaban et al. (2004).
- Persisters do not pass their tolerance to their progeny, and progeny do not inherit any greater tendency to be persisters. That is, persisters do not appear to be genetic variants. See for example Keren et al. (2004A) and Balaban et al. (2004).
- It is observed that persister cells apparently grow slowly or not at all in the presence of antimicrobial agent. See for example Balaban et al. (2004).
- Persister cells demonstrate tolerance upon exposure to multiple antimicrobial agents. That is, persister cells with respect to one antimicrobial agent can also

be tolerant upon exposure to different antimicrobial agents. As an example see Sufya et al. (2003), where survivors (from an *E. coli* batch culture) of a tetracycline challenge were also tolerant to ciprofloxacin and quaternary ammonium compound.

- Bacterial cultures demonstrate biphasic killing patterns in response to antimicrobial challenge. It has been suggested that this plateauing is a consequence of the presence of persisters. See for example Balaban et al. (2004).
- In continuous culture experiments, persister fractions are observed to increase with decreasing dilution rates, see Sufya et al. (2003).
- One of the more puzzling observations is that changes in the population fraction of persister cells are growth-phase dependent – generally, though not always, population increases do not occur until the later stages of the log phase or even until the stationary phase. For example, Keren et al. (2004A) observed an increasing ratio of persister to total cell numbers over time in *E. coli*, *P. aeruginosa*, and *S. aureus* batch cultures. In Balaban et al. (2004) the authors went as far as to posit that differences in onset of persister cell increases between different strains of *E. coli* imply the existence of more than one type of persister.

It been suggested that that persistence is a phenotypic phenomenon. Balaban et al. (2004), Roberts & Stewart (2005), Cogan (2006), propose models in which cells are able to switch in and out of a protected, slow- or non-growing persister state with probabilities that are dependent possibly on environmental conditions. In this paper, based on observations of microbial senescence (Ackerman et al., 2003; Barker & Walmsley, 1999; Mortimer & Johnston, 1959; Stewart et al., 2005), we instead propose an alternative simple mechanism that can explain all of the above mentioned properties.

The standard view of microbial cell division, at least for symmetric dividers, has been that a given cell (the mother cell) splits into two essentially identical, youthful daughter cells. However Stewart et al. (2005) demonstrate that, even in symmetric dividers, the mother cell retains its identity. That is, splitting is functionally asymmetric. During cell division the mother cell spawns one youthful daughter cell while itself remaining in the population, having aged in the process, see Figure 1. Stewart et al. (2005) show that the mother cell shows increasing senescence over the course of a number of cell divisions in the form of slowing growth rate.

In introducing this idea of senescence, we make only three essential assumptions:

1. bacterial cells age (here age is based on senescence, see e.g. Fig. 1, rather than the traditional 0 to 1 cell cycle model, e.g. Webb, 1989),
2. older cells are more tolerant than younger cells of antimicrobial challenge, and

3. growth manifests as production of new cells. That is, upon division one progenic cell inherits the effects of age while the other does not (Fig. 1).

We thus regard older cells to be the persisters. In fact, one might well label senescent cells to be a separate persister phenotype (as opposed to a youthful phenotype) though we do not stress this interpretation.

The second assumption could be a consequence for example of decreased growth rate but the particular mechanism does not really matter for our results. (The genetic bases for senescence and persistence are just beginning to emerge; see for example Nystrom, 2005, Vásquez-Laslop et al., 2006, and Spoering et al., 2006.) In reference to the third assumption, we can interpret cell division as a new, youthful cell being born from an old one.

We make the following additional non-essential specific assumptions for definiteness:

4. production rate of new cells decreases with age but remains greater than zero,
5. cell death occurs at a constant rate,
6. a given concentration of applied antimicrobial will kill cells of sufficiently young age but will not affect older cells, and
7. substrate usage depends on age and concentration, but in a separable way.

These extra assumptions matter in the details but do not affect the qualitative results that we report with the exception that a non-zero growth rate (in assumption 4) is necessary in order to enable persister cells to repopulate after antimicrobial application. For consistency with the notion of decreasing activity, we suppose that older cells grow more slowly than younger cells although this assumption is again not really necessary here; rather the focus is on senescence as a mechanism for tolerance.

2 Theory

2.1 Microbial rates

See Table 1 for a listing of variables and parameters. We define $b(a, t)\Delta a$ to be the size of the bacterial population (in cfu) between ages a and $a + \Delta a$ at time t , and $c(t)$ to be the growth media concentration at time t . Following observations reported by Stewart et al. (2005), we suppose that cells senesce at a linear rate in time; other functional forms of age-increasing senescence could be used instead. In particular for a parameter λ that we will call the senescence time, we define $r_S(a, c(t))$, the substrate usage rate per cfu at time t of cells of age a , by

$$r_S(a, c(t)) = \begin{cases} k_S[(1 - a/\lambda) + \xi]c(t), & a \leq \lambda, \\ k_S\xi c(t), & a > \lambda, \end{cases}$$

where k_S is a first-order rate constant and ξ is a base usage factor.

Similarly, we define $r_X(a, c(t))$, the new cells birth rate at time t from cells of age a , by

$$r_X(a, c(t)) = Y_S r_S(a, c(t)) \quad (1)$$

where Y_S is a yield coefficient. Additionally, by assumption 5, the death rate function r_d takes the form

$$r_d = k_d$$

where k_d is a death rate constant. Finally, functional forms for antimicrobial application will be made below.

We stress that these choices are made for simplicity and for consistency with available data from the literature. The only essential condition we require is that the applied antimicrobial agent exhibit decreasing potency as cells age (see below). For example, age-dependence in r_S and r_X is unnecessary. Conversely, r_d could be made functionally dependent, for example, on a , $c(t)$ or λ if so desired.

2.2 Age Structure

A mathematical description of age structure was first introduced in Lotka (1907), McKendrick (1926), and many such representations have been used since. Here we define $b(a, t)$ to be the bacterial population density of age a at time t , and $c(t)$ to be the available substrate concentration at time t . The equation governing b is then

$$\frac{\partial b}{\partial t}(a, t) + \frac{\partial b}{\partial a}(a, t) = -k_d b(a, t) \quad (2)$$

where the term on the right-hand side reflects cell death. As previously mentioned, the death coefficient k_d can be expected, in general, to depend on c and a ; we suppress such dependence here for simplicity as it does not affect our results in a qualitative way.

Equation (2) is valid for $0 < a < \infty$. To obtain an equation for $b(a = 0, t)$, i.e., for the new cells at time t , we observe that such cells are “born” at time t from the existing population $b(a, t)$, $a > 0$. For example, the subpopulation of cells between ages a and $a + \Delta a$ produces $r_X(a, c(t))b(a, t)\Delta a$ new cells. Summing then over the entire existing population at time t , we obtain

$$b(t, 0) = \int_0^\infty r_X(a, c(t))b(a, t)da. \quad (3)$$

A similar equation applies for the substrate concentration:

$$\frac{dc}{dt}(t) = - \int_0^\infty r_S(a, c(t))b(a, t)da. \quad (4)$$

Equations (2)-(4) are supplemented by initial conditions $b(0, a) = b_0(a)$ for some supplied initial population distribution $b_0(a)$, and $c(0) = c_0$ for some supplied initial

concentration c_0 . For discussion of mathematical issues involved in age-differentiated systems such as the one considered here, see e.g. Cushing (1998), Webb (1985).

We write (see assumption 7) $r_S(a, t) = k_S s(a) c(t)$ where

$$s(a) = \begin{cases} 1 - a/\lambda + \xi, & a \leq \lambda, \\ \xi, & a > \lambda, \end{cases}$$

($s(a)$ is the senescence factor) and so define a senescence-weighted total bacteria population

$$B(t) = \int_0^\infty s(a) b(a, t) da.$$

Then (2)-(4) become

$$\frac{\partial b}{\partial t} + \frac{\partial b}{\partial a} = -k_d b, \quad a > 0, \quad (5)$$

$$b(t, 0) = Y_S k_S c B, \quad (6)$$

$$\frac{dc}{dt} = -k_S c B, \quad (7)$$

with initial conditions $b(0, a) = b_0(a)$, $c(0) = c_0$. In the computations to follow we use $c_0 = 1 \text{ kg/m}^3$ and

$$b_0(a) = \begin{cases} (10^2 \text{ cfu}) \lambda^{-1} (1 - a/2\lambda), & a \leq 2\lambda, \\ 0 \text{ cfu/hr}, & a > 2\lambda. \end{cases}$$

With this choice, the total initial population is 10^2 cfu distributed linearly in age over the age interval $[0, 2\lambda]$.

2.3 Senescence Structure

In the previous section we have identified senescence with chronological age. In fact, Stewart et al. (2005) measure age in terms of cell-divisions (as in Fig. 1) although in their experimental set up, cell-division time and chronological time are approximately proportional. To allow for cell-division based senescence, we consider a general senescence-structured population model,

$$\frac{\partial}{\partial t} b(\sigma, t) + \frac{\partial}{\partial \sigma} (v(\sigma, c) b(\sigma, t)) = -r_d(\sigma, c) b(\sigma, t), \quad \sigma > 0, \quad t > 0, \quad (8)$$

where σ is an index of senescence and the function $v(\sigma, c)$ is the rate of increasing senescence per time. Senescence may be determined by chronological age (see previous section with $\sigma = a$, $v = 1$) or in some other manner. In particular, we can identify senescence σ to be proportional to the number of cell-divisions as in Figure 1 in which case $v(\sigma, t) = \phi r_X(\sigma, t)$ where g is as given previously, see (1), and ϕ is a constant of proportionality.

For ease of calculations we change variables from σ to a to obtain

$$\frac{\partial}{\partial t}b(a, t) + \frac{\partial}{\partial a}b(a, t) = -\tilde{k}(\sigma, c)b(a, t), \quad (9)$$

$$\frac{\partial}{\partial a}\sigma(a, t) = v(\sigma, c), \quad (10)$$

where $a > 0$, $t > 0$, $\sigma(0, t) = 0$, and $\tilde{k}(\sigma, c) = k(\sigma, c) + \frac{\partial}{\partial \sigma}v(\sigma, c)$. The birth condition becomes

$$b(0, t) = \int_0^\infty v(\sigma(a, t), c(t)) r_X(\sigma(a, t), c(t)) b(a, t) da.$$

2.4 Chemostat Model

In addition to batch culturing, we consider a chemostat system for which equations (5)-(7) become,

$$\frac{\partial b}{\partial t} + \frac{\partial b}{\partial a} = -(k_d + D)b, \quad a > 0, \quad (11)$$

$$b(t, 0) = Y_S k_S c B, \quad (12)$$

$$\frac{dc}{dt} = -k_S c B + D(C_0 - c), \quad (13)$$

where D is the chemostat dilution rate and C_0 is the reservoir substrate concentration.

2.5 Antimicrobial Application

We include the effect of applied antimicrobial consistently with assumption 6: a given antimicrobial concentration d applied at time t to the bacteria population results in killing at rate γ of sufficiently young (and hence susceptible) cells and does not effect older (and hence tolerant) cells. We assume for definiteness and consistency with our senescence assumption that tolerance age increases linearly with antimicrobial concentration. Other choices of age dependence, as long as they are monotone in senescence, can be made. While Stewart et al. (2005) report 1-2% decay in growth rate per generation, this is an average decay and presumably there is some distribution in senescence rate. This effect could be included in the model but for sake of simplicity we do not do so here. This omission might result in overestimate of persistence numbers (perhaps only the most senescent outliers should be considered as persistent) but we don't believe there are other important qualitative consequences.

Then equation (5) is replaced by

$$\frac{\partial b}{\partial t} + \frac{\partial b}{\partial a} = -(k_d + r_K(a, d))b, \quad a > 0, \quad (14)$$

with killing rate function r_K defined by

$$r_K(a, d) = \begin{cases} k_K, & a \leq \delta d, \\ 0, & a > \delta d. \end{cases} \quad (15)$$

where k_K is a killing rate coefficient. (The corresponding change in equation (8) for the cell-division version of senescence is similar.) Here δ is an adjustable tolerance coefficient. For a given antimicrobial concentration d , small δ means that cells become tolerant at a relatively young age, and large δ means that cells become tolerant at a relatively old age. For numerical reasons, we slightly smooth the discontinuity in r_K in the results reported below. Based on (15) then, for a given antimicrobial concentration d , cells of age δd or greater result in persistence.

3 Methods

3.1 Parameters

The model we describe contains parameters d , k_d , k_S , k_K , Y_S , δ , λ , ϕ , and ξ (plus two, D and C_0 for the chemostat), see Table 1. We choose representative, reasonable values, see below. Qualitative features are robust with respect to variation of these parameters within reasonable ranges. (The exception is δ for which an estimate is not available and for which variation changes the onset of persistence.) In fact, excepting δ , the only essential imposed constraints on these parameters necessary for the results we report are that $k_K > k_d$, i.e., the antimicrobial killing rate is greater than the cell death rate, that $\delta > 0$, i.e., cells become more tolerant with age, and that $\xi > 0$ so that persister cells are capable of repopulation. We set the parameter values as follows:

Growth and substrate usage parameters: for definiteness, we set the length of the log phase to be approximately 10 hr and the bacteria doubling time to be approximately 0.75 hr, resulting in approximately 13.3 doublings in the log phase. Given an initial value of 10^2 cfu, we thus obtain approximately 10^6 cfu at the end of the log phase. These constraints require $c_0 k_S \cong 2^{-13.3} \cong 10^{-6}$ and $c_0 k_S Y_S \cong 1.33 = (0.75)^{-1}$ (in units as in Table 1). We set $c_0 = 1$ kg/m³ for initial substrate concentration, and then use $k_S = 10^{-6}$ hr⁻¹cfu⁻¹, $Y_S = 1.33 \cdot 10^6$ cfu·m³/kg. (Roughly speaking, k_S^{-1} fixes the population at the end of the log phase and $(k_S Y_S)^{-1}$ determines the length of the log phase via the doubling time.) The minimum substrate usage parameter ξ is presumed to be small compared to 1, and needs to be larger than zero in order for persister cells to repopulate. Otherwise its value is unimportant. We set $\xi = 10^{-3}$. The value of the cell death rate is unimportant as well and could even be set to zero (the exception is for the continuous culture case where k_d determines the slow dilution limit persister population); we use $k_d = 0.05$ hr⁻¹.

Senescence parameters: Supposing significant senescence after about 16 generations (Stewart et al. (2005) reports 1-2% per generation) with a cell division time of 0.75 hr, we then obtain $\lambda = 12$ hr.

Antimicrobial parameters (except δ): As a typical antimicrobial dosage, we use $d = 0.01$ kg/m³ (Roberts & Stewart, 2005). As a typical antimicrobial killing rate,

we use $k_K = 10 \text{ hr}^{-1}$ (Sufya et al., 2003).

Chemostat parameters: we allow D , the dilution rate, to vary. The other parameter, reservoir concentration C_0 , does not affect our results so is arbitrary. To estimate ϕ , we require as above significant senescence after 16 cell-divisions, i.e., σ should be approximately 12 after 16 cell-divisions. Thus we set $\phi = 12/16 = 0.75$.

The only important quantity without an estimate is the tolerance age δd at which bacteria become tolerant to the antimicrobial (see equation (15)). We assume that the tolerance age be equal to λ , the senescence time, and thus $\delta = \lambda/d = 1.5 \cdot 10^3 \text{ hr} \cdot \text{m}^3/\text{kg}$. Increasing (decreasing) δ has the effect of increasing (decreasing) the tolerance age.

3.2 Numerical Methods

Equation (14) (or (9)-(10) with accompanying conditions) along with (6), (7) are solved numerically. We use a moving-grid Galerkin method in age with discontinuous piecewise linear functions post-processed to cubic splines for the approximation space in age (Ayati & Dupont, 2002) along with a step-doubling method in time, Ayati & Dupont (2005). This combination was illustrated in Section 5, Ayati & Dupont (2002), using the same code as used here.

4 Results

In Figure 2 we apply antimicrobial to a stationary phase culture, Fig. 2(a), and to a log phase culture, Fig. 2(b), and then “reculture” afterwards by removing the antimicrobial and adding fresh medium. Note the biphasic survival in each case: there is an initial sharp die-off immediately after antimicrobial application followed by a second phase of die-off of older (persistent) cells due to natural causes. We also note that the number of persisters in this example does not grow substantially until the stationary phase – this is a consequence of the delay between birth of new cells in the log phase and aging of those cells into persisters. That delay time depends on δ , our only free coefficient. Small δ results in short delay in the generation of persistent cells (i.e., growth in persister numbers even in the log phase) while large δ results in long delay in the generation of persistent cells. The growth at early times in persistence numbers seen in both Fig. 2(a) and Fig. 2(b) is solely a consequence of transients due to the initial age distribution in the inoculum. Note for example that persister numbers actually decline during the second log phase in both Fig. 2(a) and Fig. 2(b). We remark that the model predicts that most cells become persistent in late stationary phase. Late stationary phase data is scarce since all cells approach quiescence; however, data from continuous flow systems at low dilution rate (Sufya et al., 2003), which may be somewhat analogous to late stationary batch cultures, suggests that persister percentage does indeed approach 100.

Figure 3 illustrates the same computational experiment as in Fig. 2 except using cell-division based senescence rather than chronological age based senescence. Note the similarity between the two. Onset of significant persistence is somewhat later in the cell-division based case but this is in fact an artifact of the choice of proportionality constant ϕ relating cell-division rate to senescence rate ($\phi = 0.75$ in Fig. 3). Large values of ϕ for example result in earlier onset. Due to the similarity of the results between chronological age and cell-division age based senescence models, we use only the somewhat simpler chronological age model below.

In Figure 4 we present a computational version of the persister elimination experiment conducted in Keren et al. (2004A), see Figure 4 of that reference, qualitatively matching the results reported there. These authors observed that persister numbers could be driven downwards by frequent reculturing. In the present model, this phenomena occurs because only a small percentage of cells are able to survive frequent cullings and thus reach senescence. In addition to serving as a comparison test of the persistence model to experiment, our (computer) experiment serves to emphasize the point that the senescence mechanism presented here reproduces evidence that persisters are not formed in early log phase, and that by suppressing production of senescent cells, it is possible to suppress persister frequency as in Keren et al. (2004A).

In fact, the persistence elimination experiment illustrated in Fig. 4 might be considered as an approximation to a chemostat with roughly 2 hour turnover. So we also consider persister numbers in the chemostat system (11)-(13) at steady state (see Sufya et al., 2003). In particular, by setting $\partial b/\partial t = 0$ in (11) and suppressing t dependence, we obtain

$$b(a) = b(0)e^{-(k_d+D)a}.$$

Thus the persister population fraction P is given by

$$P = \frac{\int_{\delta d}^{\infty} b(a) da}{\int_0^{\infty} b(a) da} = e^{-(k_d+D)\delta d},$$

see Figure 5 (though at sufficiently high dilution rate, washout of cells exceeds the maximum specific growth rate and thus the biomass concentration, including persisters, becomes zero in steady state). We again note the qualitative match to the experimentally reported results in Sufya et al. (2003). In particular, note the rapid transition from high to low persister fraction separating the two regimes D^{-1} smaller than and larger than the persister age δd . For small D , i.e., slow dilution, persister fraction tends to a constant controlled by k_d , namely $e^{-k_d\delta d} \approx 0.55$ for the parameter values used here. For large D , i.e., fast dilution, persister fraction tends to zero. We remark that this characterization of persister fraction with respect to dilution rate, see Fig. 5, is independent of the details of substrate usage. That is, it is independent of equations (12) and (13). It is also independent of the form of the senescence factor.

5 Discussion

The explanation of persistence as a symptom of senescence is an attractive one. Tolerance due to senescence, possibly because of reduced growth rate, explains in a simple manner the persistence characteristics listed in the Introduction:

- Offspring of old cells are of course young, and able to quickly repopulate.
- Offspring of old cells are young and active cells, and hence do not inherit low activity tolerance, i.e., are not themselves persistent.
- Old cells grow slowly.
- Activity is independent of choice of antimicrobial agent so that inactive cells can demonstrate tolerance to multiple agents.
- In the presence of an antimicrobial agent, non-persisters are killed quickly followed by slow die-off of persisters, i.e., biphasic behavior occurs.
- Decreasing the dilution rate in a continuous culture allows more cells to age longer before washout, hence increasing the persister fraction.
- Increase in persistence numbers can appear to depend on growth phase. In particular new cells are, obviously, young cells and so log phase growth does not affect persistence numbers until enough time has passed for those new cells to age sufficiently. Hence persistence numbers do not significantly increase until later in the log phase or even the stationary phase (and may actually decrease before).

All of these observations are qualitative properties of our model depending on assumptions (1)-(3) and, we believe, essentially independent of the particular choices made in the other assumptions.

A number of authors have previously suggested persisters to be switching phenotypic variants (e.g. Balaban et al., 2004, Cogan, 2006, Kussell et al., 2005, Roberts & Stewart, 2005, Sufya et al., 2005, Wiuff et al., 2005), that is, that persisters are cells with the same genome but with different sets of genetic expression as “normal” cells, and that a given cell can switch back and forth between the two states. The resulting phenotype switching model consists then of cells transiting between persister and non-persister phenotypes. We regard asymmetric aging as an alternative pathway to the persistence phenomenon. Whereas the persister cell concept invokes, to many, binary switching or differentiation of cells between protected and non-protected states, the aging concept in contrast posits a distribution of cell ages in a population and a correlation between age and susceptibility. Beyond thinking of aging as a mechanism to generate persister cells, we suggest asymmetric aging as a mechanism to generate distributed phenotypes (antimicrobial susceptibility or some other) within a population.

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8 Tables

Table 1: Variables, Parameters, and Functions			
Symbol	Quantity	Units	Value
a	age	hr	
$B(t)$	weighted population at time t	cfu	
$b(a, t)$	population per age at age a and time t	cfu/hr	
$b_0(a)$	initial population per age at age a	cfu/hr	
C_0	reservoir substrate concentration	kg/m ³	variable
$c(t)$	substrate concentration at time t	kg/m ³	
c_0	initial substrate concentration	kg/m ³	1
D	chemostat dilution rate	hr ⁻¹	variable
d	antimicrobial dosage	kg/m ³	0.01
k_d	nominal cell death coefficient	hr ⁻¹	0.05
k_K	cell killing coefficient	hr ⁻¹	10
k_S	substrate usage coefficient	hr ⁻¹ ·cfu ⁻¹	10 ⁻⁶
$r_K(a, d)$	killing rate function	hr ⁻¹	
$r_S(a, c)$	substrate usage rate function	kg·m ⁻³ ·hr ⁻¹ ·cfu ⁻¹	
$r_X(a, c)$	cell growth rate function	hr ⁻¹	
$s(a)$	senescence factor	-	
t	time	hr	
$v(s, c)$	senescence increase rate	senescence/hr	
Y_S	cell yield coefficient	cfu·m ³ /kg	1.33 · 10 ⁶
δ	tolerance coefficient	hr·m ³ /kg	1.5 · 10 ³
λ	senescence time	hr	12
ϕ	senescence proportionality constant	senescence units	0.75
σ	senescence	senescence units	
ξ	minimum substrate usage factor	-	10 ⁻³

9 Figures

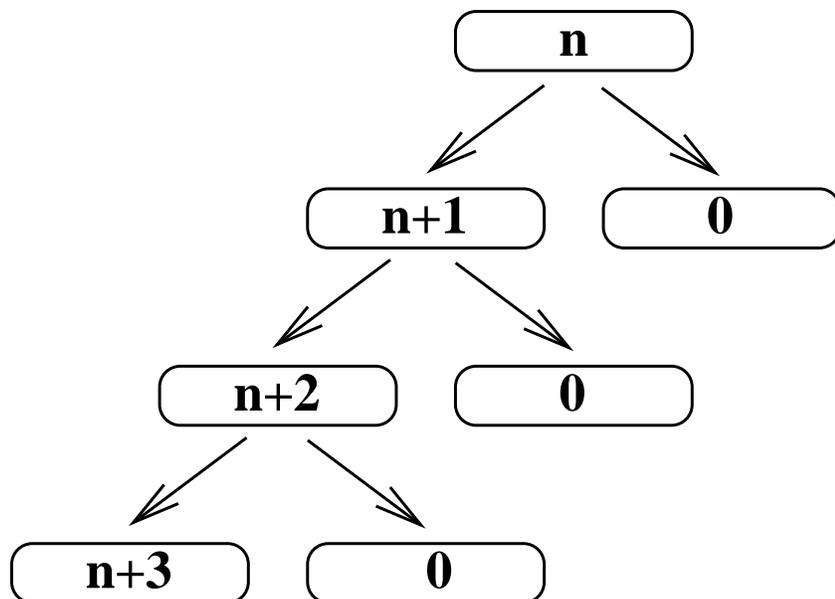


Figure 1: A mother cell (top) divides at time intervals of, say, length 1. Upon each division, the mother cell has aged by one time unit and has produced a youthful daughter cell of age 0.

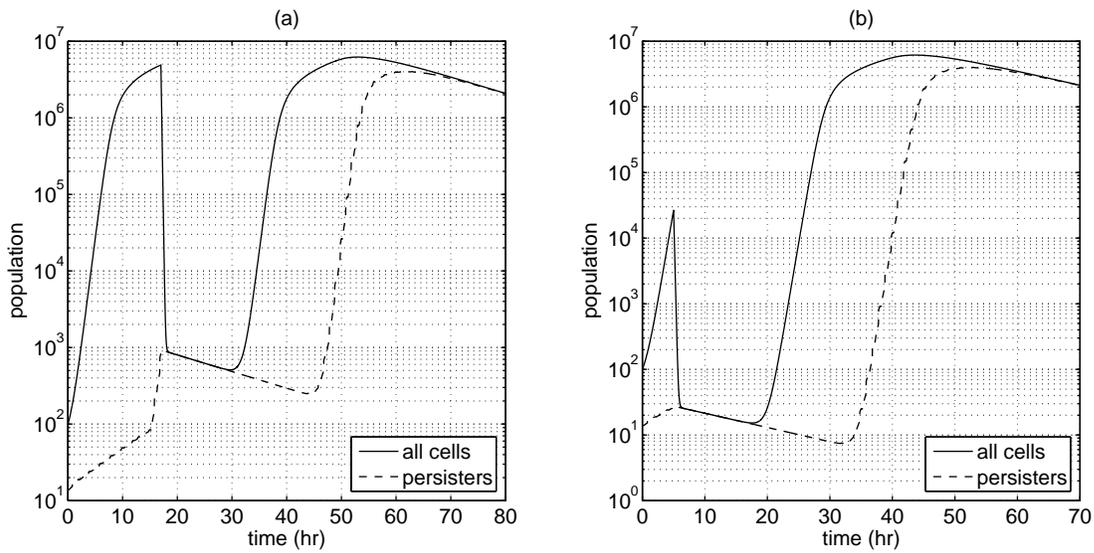


Figure 2: Exposure of a batch culture to antimicrobial with senescence determined by chronological age. (a) antimicrobial is applied during stationary phase at $t = 17$ hrs and removed at $t = 27$ hrs at which time surviving cells are recultured at the $t = 0$ hrs nutrient level. (b) antimicrobial is applied during log phase at $t = 5$ hrs and removed at $t = 15$ hrs at which time surviving cells are recultured at the $t = 0$ hrs nutrient level.

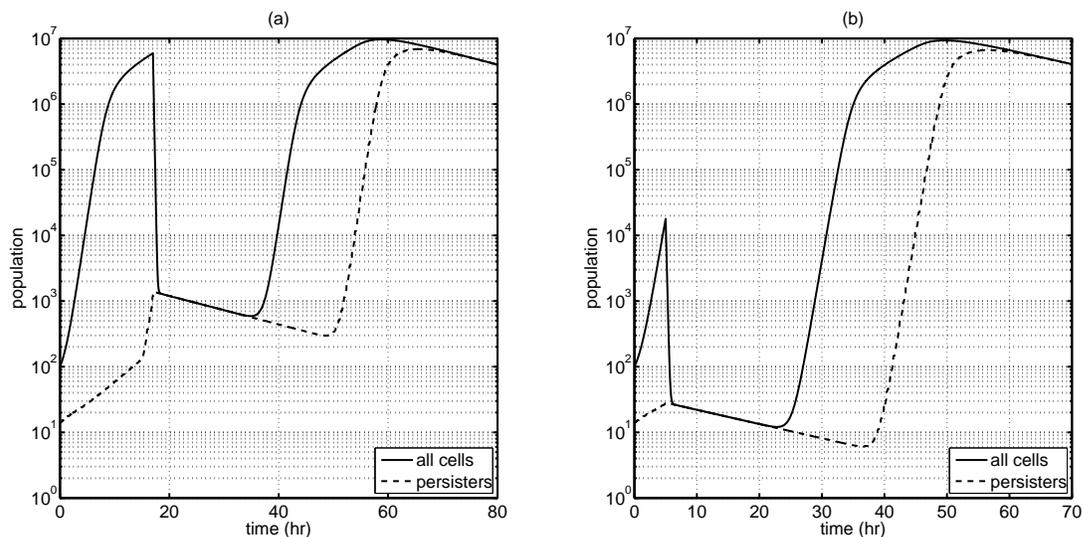


Figure 3: Exposure of a batch culture to antimicrobial as in Figure 2 except that senescence is proportional to cell division number rather than chronological age. (a) antimicrobial is applied during stationary phase at $t = 17$ hrs and removed at $t = 27$ hrs at which time surviving cells are recultured at the $t = 0$ hrs nutrient level. (b) antimicrobial is applied during log phase at $t = 5$ hrs and removed at $t = 15$ hrs at which time surviving cells are recultured at the $t = 0$ hrs nutrient level.

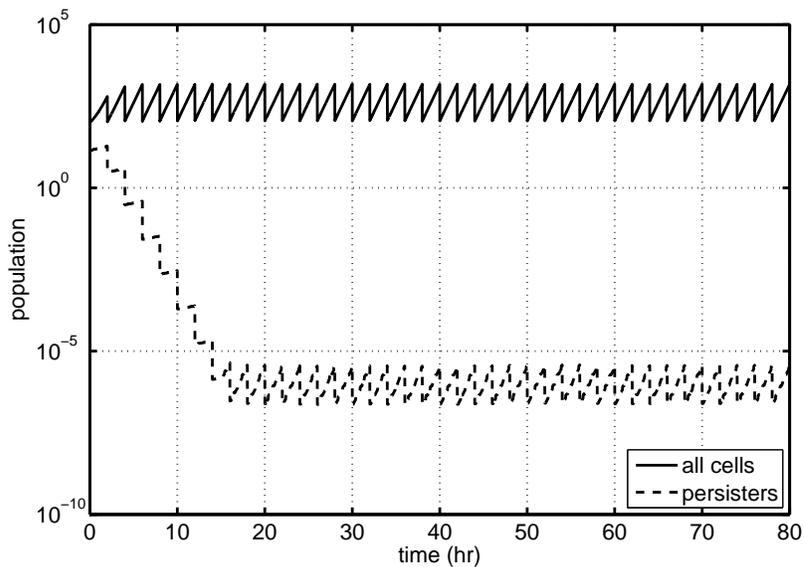


Figure 4: Bacteria batch culture, diluted down to 10^2 organisms every two hours. Solid curve is the total population and dashed curve is the persister population. See Keren et al. (2004A), Fig. 4.

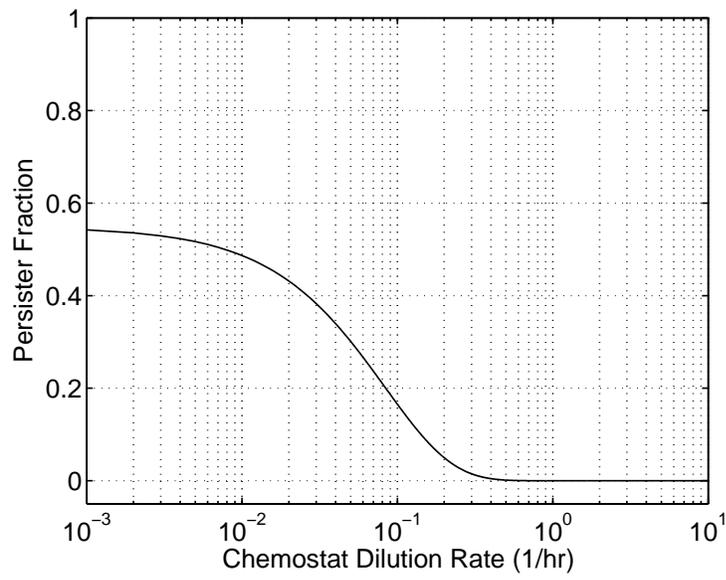


Figure 5: Fraction of persisters in a steady state chemostat as a function of chemostat dilution rate.