Simulation notes 3

Steven Court

May 17, 2016

1 Probability of reaching final well

I wanted to check how easy it was to increase or decrease the likelihood that the experiments would reach the final well, even for the maximum concentration of 20 000 ng/ml Bartek used in his initial experiments. The reason I was checking this was because for the simulations I ran initially, with $P_{gyrA1} = P_{gyrA2} = P_{parC}$ scaled to match the expected number of mutations per well in the experiments, and $P_{marR} = P_{acrR} = 100P_{gyrA1}$ I saw 50/50 runs reach the final well, unlike in Bartek's experiments where none did.

- First thing I checked was lowering the chances of marR mutations. Reducing the multiplication factor from 100 to both 10 and 1, I saw 27/30 runs reach the final well in both cases. I don't know how much of a difference I expected to see. To reach the final well with this concentration profile we need 1 of 5 genotypes to have evolved: 10111, 11100, 11110, 11101, 11111, where the order is gyrA1, gyrA2, parC, marR, acrR. Maybe above a certain point, having a higher chance of marR and acrR mutations doesn't help so much, since in every one of these genotypes we need a parC with at least 1 or both of the gyrA mutations. (One other thing I should mention is that I have multiplied the MIC values in Marcusson2009 by 2, since they have an MIC of 16 ng/ml whereas Bartek's measured value appears to be > 35ng/ml. I suspect this isn't important but haven't check it).
- I checked that increasing the carrying capacity (thus lowering the amount we need to scale P_{gyrA1}) didn't look very different. For $K = 10^7$ and $P_{marR} = P_{gyrA1}$ I saw 8/9 runs still reach the final well. Each run takes 24 hours for this number of bacteria. I think I could make the code quite a bit more efficient by changing the way I implement the genotypes, maybe $K = 10^8$ would be possible.
- I have seen bp mutation rates stated between 10^{-9} and 10^{-10} for E. coli in the literature. Reducing γ by a factor of 10 to 10^{-4} made a noticeable difference. For $P_{marR} = 100P_{gyrA1}$ 36/60 runs reached the final well (for $a_{max} = 20000$ ng/ml) and using $P_{marR} = P_{gyrA1}$ now had a more noticeable affect, lowering it to 14/60 runs reaching the final well.

1.1 Estimating probabilities of mutation occurrences in experiment

I don't know how useful these numbers are, since they neglect selection and growth, but maybe they're insightful; I was surprised how large they are. If we assume that the gyrA1 mutation is a single bp, and that the bp-mutation probability is 10^{-9} per replication, then the probability of seeing a mutated gyrA1 in a single replication event is $P_{\text{gyrA1}} = 10^{-9}$. This means, roughly, that in a single well with carrying capacity K (thus expecting K replication events) the probability that we get 1 or more gyrA1 mutation events in this well is:

$$P_{>1\text{gyrA1/well}} = 1 - P(\text{no gyrA1}) \quad (1)$$

$$= 1 - (1 - P_{\text{gyrA1}})^K = 0.095 \quad (2)$$

for the experimental $K = 10^8$. So, if we look at the case for which the maximum antibiotic concetration is 20,000 ng/ml, the MIC of the WT bacteria is not actuallly reached until well 9 using the exponential profile in Bartek's original experiments. Ignoring death, this means that the probability that a gyrA1 mutation occurs before the MIC is reached is just 1 minus the probability that no gyrA1 mutation occurs in each of the earlier wells; i.e. $1-(1-0.095)^9 = 0.59$, thus a 60% chance. If the chances of a gyrA2 and parC mutation are the same as gyrA1, this means that the probability that at least 1 of each has occurred by the 9th well is still relatively high: $0.59^3 = 0.20$, though obviously this is just 3 random events, not necessarily in the same bacteria (though selection in the later wells is stronger, making fixation and thus multiple mutations per genome much more likely). The probability that we have gyrA1 *or* gyrA2 before we hit the WT MIC is $0.59 + 0.59 - 0.59^2 = 0.83$, so it is very likely that we get one of these important mutations which would allow us to colonise up to wells 16 or 14 respectively, without any further mutations.

Note that in the simulations I am currently increasing the mutation rate with ciprofloxacin concentration via the form:

$$\gamma(a) = \gamma_{WT}\left(1 + \left[\frac{Xa}{MIC_{WT}}\right]^{Y}\right) \tag{3}$$

which will increase these probabilities further. X and Y are arbitrary parameters, currently 15 and 1.5 respectively, which means that $\gamma(a = 0.5\text{MIC}) = 20\gamma_{\text{WT}}$ and $\gamma(a = \text{MIC}) = 58\gamma_{\text{WT}}$. I impose a hard upper cutoff of $\gamma_{max} = 200\gamma_{WT}$ but I have seen that mutation rates in the literature can vary over 4 orders of magnitude (though these were ΔlacZ and ΔmutS hypermutator strains I think).

What this means is that in our simulations, if a gyrA1 mutant is present it can colonise up to well 16. With the exponential concentration profile with a maximum of 20,000 ng/ml, in well 12 we have already reached the maximum mutation rate of $\gamma_{max} = 200 * \gamma_{WT} = 200 * 10^{-3} = 0.2$ using Eq. 3. So the probability of seeing one or more gyrA2 events in well 12 is now, ignoring death, $1 - (1 - 200 \times 10^{-9})^{K} \sim 1$. So what I think this means is that the outcome of my simulations was being dominated by what happened in the few wells before the MIC-containing well (whether or not we got a gyrA1 or gyrA2 mutation).

I hadn't calculated this before – perhaps I shouldn't be increasing the mutation rate so much? Or maybe a base rate of $\gamma_{WT} = 10^{-4}$ is more realistic? Or maybe we should be surprised that the experiments never did reach the final well and ask why? Also, in reality, there is a limit to the benefit of an increased mutation rate as cells will accumulate more deleterious mutations. Should the probability of death during replication also be linked to the mutation rate or the number of accumulated mutations?

1.2 Experimental facts

I think there are a number of things it would be interesting to know about the experiments, though I don't know how easy it would be.
(1) How mutation rate depends on (i) concentration of ciprofloxacin (ii) the individual mutant strains. For example, taking one of the most resistant strains and growing it at 1/4 of its MIC (maybe still > 1000 times the WT MIC), is the the mutation rate hugely increased or not? Bartek has measured a 10-fold increase for 0.5 MIC concentrations, for the wildtype. In [2] they state that from the studied strains, the spontaneous mutation rate varied by 2 orders of magnitude between strains. SOS response can apparently increase bp-mutation rate by 4 orders of magnitude [1].
(2) Does ciprofloxacin concentration affect motility? Do the resistant bacteria still filament in the later wells and is this reducing motility to zero? E. coli exposed to 1-10×MIC filamented [3] – will surely affect motility once cells start to deviate from rod shape. In Bob Austin's paper [1] 99.5% of cells filament at low concentrations (as low as 0.125×MIC). The paper [4] states that cells did not lose their motility for sub-inhibitory concentrations but no quantitative measurements were made. Experimentally can we see if (i) the wildtype wavespeed was affected in different, uniform sub-inhibitory concentrations; (ii) if the form of this dependence is gradual or a sharp cut-off; (iii) to see if some of the resistance-evolved strains behave the same, or have different wavespeeds in the presence / absence of ciprofloxacin. This behaviour could be important in understanding the results of the experiments.

2 Using experimental LB growth data

Quick reminder of how simulation works: In the simulation, each well is given an initial amount of food and each replication event is associated with the consumption of 1 food unit. The birth rate for an individual bacteria in well i is calculated as:

$$br[i] = \text{growth}() * \text{growth\_function}(\frac{\text{food}_\text{i}}{\text{food}_0}) \tag{4}$$

where the growth() function just returns the maximum growth rate, 2.0, and the growth_function() method returns what it is given, currently the ratio of food left in the well (equivalent to $K - N$) to the initial food in the well (equivalent to the carrying capacity K). Hence growth rate in each well is logistic growth:

$$g = N * br[i] = N * 2 * \left(1 - \frac{N}{K}\right). \tag{5}$$

There is no death rate currently implemented, rather upon each replication event there is a ciprofloxacin-dependent probability that the cell does not divide but dies, given by:

$$P_d = \left(\frac{a}{\text{MIC}}\right)^2, \tag{6}$$

where a is concentration of ciprofloxacin and we force $P_d \leq 1$. This form was chosen to roughly match Bartek's experimental data.

Bartek took his OD data for growth on an LB medium and calculated a growth rate vs N/K from it so that I could implement it in the simulation. Figure 1 shows the form of this growth compared to the original logistic form while Figure 2 shows the population growth of a single well and the 24-well array.

Using Bartek's data I no longer implement food explicitly; the population is limited only on a per-well basis (depending on it's population which may fluctuate due to migration). This new form for the growth rate changes the shape of the travelling waves (and wavespeed) from the case of logistic growth. Figure 3 shows the form of the travelling wave for both growth functions for the same set of parameters.

I checked to see the dependence of wavespeed on carrying capacity, (200 runs for $K = 10^3$–10^6 but for $K = 10^7$ the simulations took much longer than I anticipated so the number of runs plotted is about 75). Figure 4 shows how the wavespeed increases with K, but I'm not sure what I should be comparing this to. You said in the last meeting something like:

$$v = 2\sqrt{Dr} \pm c/(\ln K)^2, \tag{7}$$

which I have "fitted" by eye in Figure 4 using $v = 0.82 - 12/(\ln K)^2$. In the simulation, $D = 1.1$ mm^2/h while the growth rate $r = 2.0$ h^{-1}, giving $2\sqrt{Dr} = 2.97$ mm/h. The well width was 3.25 mm and hence we have $2\sqrt{Dr} = 0.91$ wells/h. (Is this true in 3 dimensions?) Note that this does not take into account the channels we have that are connecting the wells in our simulations - obviously the wavespeed will depend on the channel dimensions - so I think this value of 0.91 is the theoretical upper limit for the parameters used. Check this by making channels as large as possible.

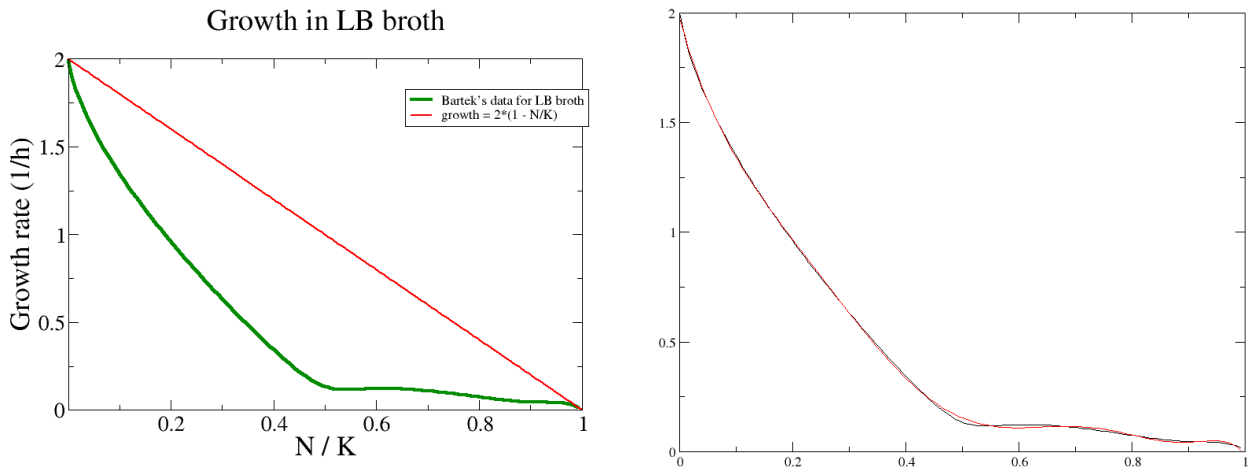
References

[1] Julia Bos, Qiucen Zhang, Saurabh Vyawahare, Elizabeth Rogers, Susan M. Rosenberg, and Robert H. Austin. Emergence of antibiotic resistance from multinucleated bacterial filaments. *Proceedings of the National Academy of Sciences of the United States of America*, 112(1):178–83, 2015.

[2] Patricia Komp Lindgren, Asa Karlsson, and Diarmaid Hughes. Mutation rate and evolution of fluoroquinolone resistance in Escherichia coli isolates from patients with urinary tract infections. *Antimicrobial agents and chemotherapy*, 47(10):3222–3232, 2003.

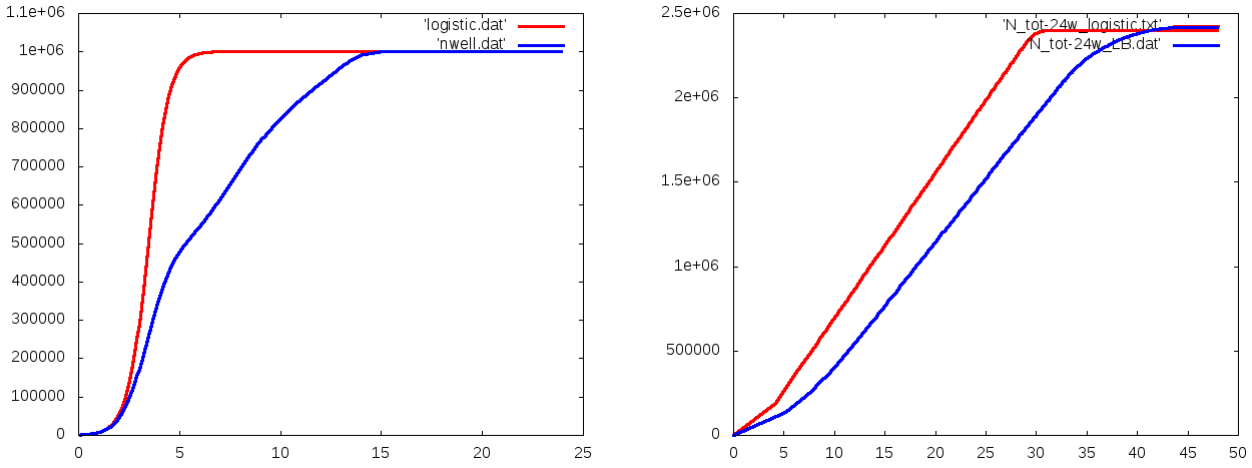
[3] D J Mason, E G Power, H Talsania, I Phillips, and V a Gant. Antibacterial action of ciprofloxacin . These include : Antibacterial Action of Ciprofloxacin. 39(12):2752–2758, 1995.

[4] Dorota Wojnicz and Dorota Tichaczek-Goska. Effect of sub-minimum inhibitory concentrations of ciprofloxacin, amikacin and colistin on biofilm formation and virulence factors of Escherichia coli planktonic and biofilm forms isolated from human urine. *Brazilian Journal of Microbiology*, 44(1):259–265, 2013.



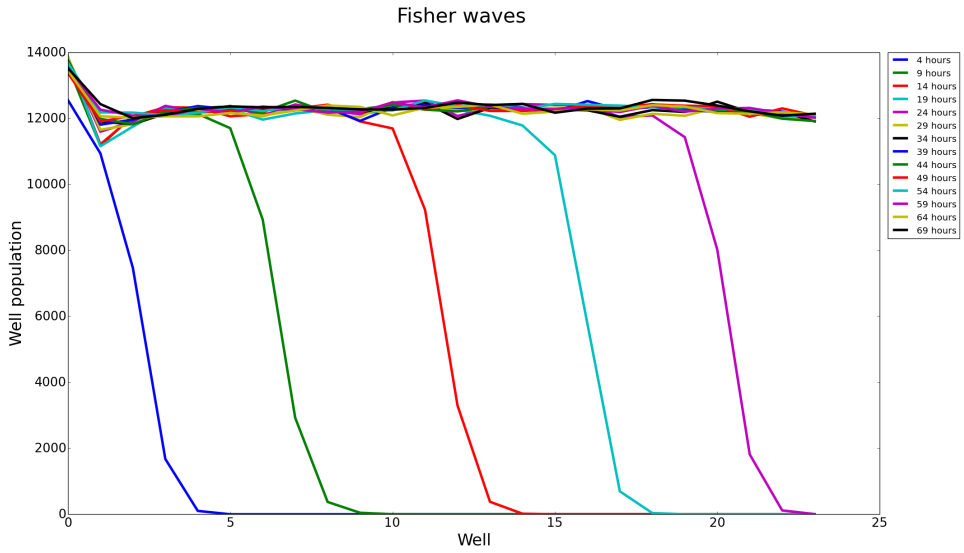
(a) (b)

Figure 1: (a) Experimental E. coli growth rate in LB growth compared to standard logistic growth. (b) Polynomial fit to data using xmgrace: $a_0 + a_1x + a_2x^2 + ... + a_9x^9$, where $a_0 = 1.97404, a_1 = -9.17449, a_2 = 40.0716, a_3 = -143.678, a_4 = 325.762, a_5 = -679.34, a_6 = 1451.89, a_7 = -2072.97, a_8 = 1519.2, a_9 = -433.754$. (Though I'm not currently using this, but instead using Bartek's raw data).

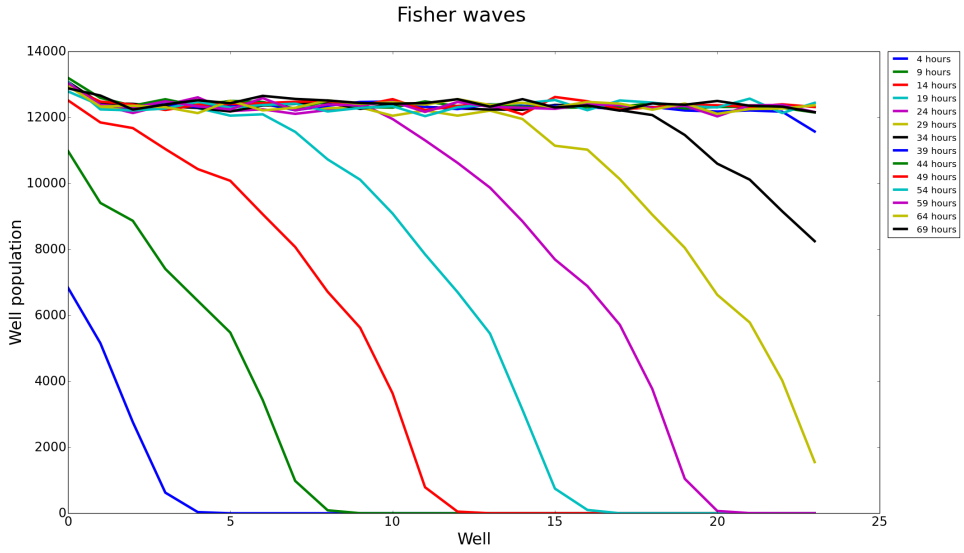


(a) (b)

Figure 2: (a) Population growth in a single well for logistic (red) and experimental LB (blue) growth rates. (b) Growth of total population N_{tot} across all 24 wells.



(a)



(b)

Figure 3: Shape of travelling waves for original logistic growth (a) and when using Bartek's experimental LB growth data (b). Both use a diffusion constant of 1.5 and carrying capacity of 10^5 per well. In these runs, the wavespeed for logistic growth (a) is 0.9 wells/h and LB-growth (b) is 0.8 wells/h.

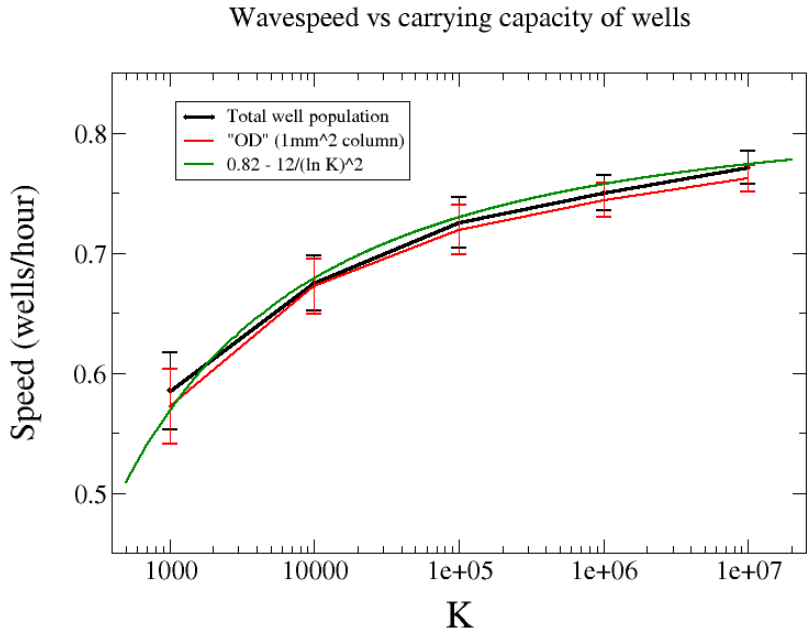


Figure 4: Average wavespeed (wells/h) vs carrying capacity K. Error bars are standard deviations from 200 experiments (except for the $K = 10^7$ point which is 75). The green line is a fit I guessed by trial and error using the form of Eq. 7 – it's quite a good fit!