Prolonged culture and long lasting infections select for poorly transmitted bacterial variants — Supplementary materials S2

November 30, 2018

In vitro kinetics of the 34 isolates of the collection

We assessed *in vitro* growth kinetics of the 34 isolates by measuring GFP fluorescence using a Synergy BioTeK spectrophotometer. More precisely, GFP-mediated fluorescence was estimated by measuring emissions at 535nm after excitation at 485nm. In order to control for potential autofluorescence, we also measured emissions at 625nm and computed the log-transformed ratio of emissions at 535nm and 625nm. In the following, we will consider this quantity as an estimate of GFP emission intensity.

For each experiment, 24 hours precultures were first initiated from cryotubes and incubated in 200 μL LB in 96-wells clear-bottom microplates (Greiner) at 28°C with 20μg Kanamycin per mL. Each of the 34 variants was randomly assigned to two to three positions in the plate and a single well filled with sterile culture medium was used as a negative control.

After 24 hours, precultures were centrifugated and rinsed in sterile LB. We then

measured GFP emission intensity and performed a dilution in order to compensate for differences in fluorescence among the wells of the microplate. From then, we transferred 20μL of a 10^{-3} dilution of each preculture into approx. 180μL of fresh LB with Kanamycin added. The culture microplate we obtained was then placed in the spectrophotometer, at 28°C with shaking, and measurements were performed every 15 minutes for 91 hours. We ran five replicate experiments.

In addition to fluorescence measurements, we estimated bacterial density by spreading appropriate dilutions of cultures (typically 10^{-5} or 10^{-6}) onto NBTA plates with 50μg Kanamycin per ml. Colonies were counted after 48 hours incubation at 28°C. This was performed first at the onset of the experiment, in order to both check that cell densities were the same in all wells of the culture microplate and estimate inoculum size. This was also performed after the 91 hours of *in vitro* cultivation, so that we can check that differences in fluorescence do relate to differences in cell density. From the spectrophotometer measurements, we estimated the time at which we detected bacterial growth in each well. This was done by first estimating the average GFP intensity over the first 3 hours of the kinetics, a time at which bacteria have not yet multiplied, and adding an arbitrary value of five percent to this average value. We obtained this way a threshold intensity above which we consider that fluorescence is due to the presence of bacteria. For each well of the culture microplate, we then estimated the time at which the GFP intensity has reached the threshold value. We will further refer to this computation as time lag.

From the same experiments, we computed the average number of Colonies Forming Units (CFU) per mL, from counts on NBTA plates after the 91 hours of cultivation. In most cultures, number of CFU per mL could be estimated for two distinct dilutions. In this situation, we averaged the two estimates. We used these NBTA plates to estimate the proportion of red CFU for each of the 34 variants.

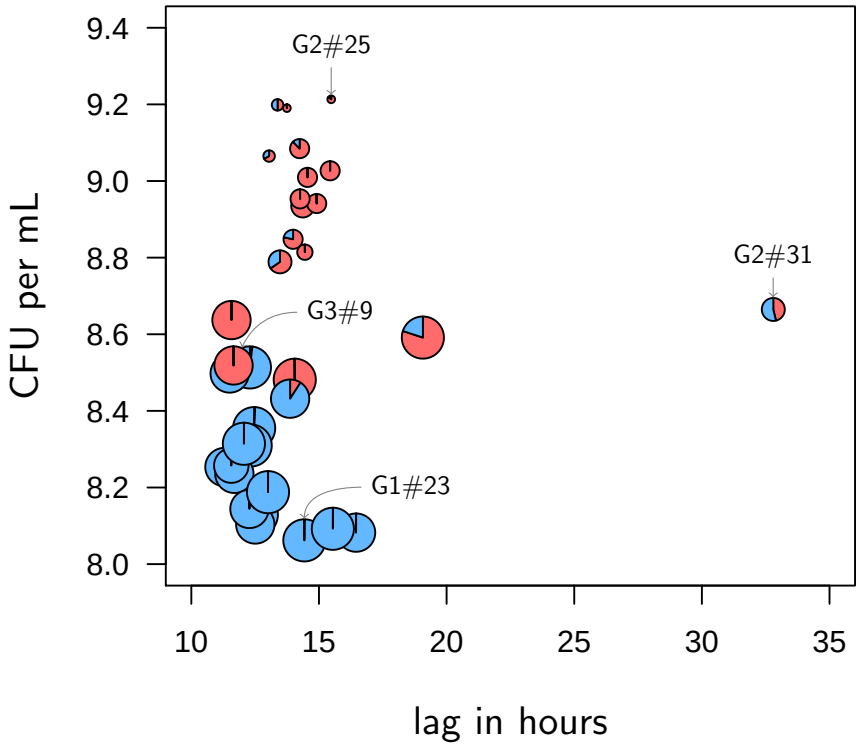

Figure S2-1: Lag in hours and density in log CFU per mL for each of the 34 isolates of the collection.

For each of the 34 isolates, following the procedure described above, we obtained from 12 to 15 estimates of time lag and from 9 to 12 estimates of CFU numbers. We summarized all these data by computing average values which are represented in the figure below. In this figure, G2#31 clearly stands apart as the isolate with the longest lag. Overall, this figure also illustrates that group 2 and to a lesser extent group 3 variants reach higher densities than group 1 variants. These estimations also demonstrate that group 2 isolates start growing later (Wilcoxon test: $p = 0.004$) but reach higher CFU per mL (Wilcoxon test: $p = 7.7e - 8$) than group 1 isolates. Group 3 variants also reach higher CFU per mL than group 1 (Wilcoxon test: $p = 0.00155$) but have a similar lag.