Laboratory of Directed Evolution

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Studying adaptive processes in real time has tremendously improved during the past decades. However, there are still limits when it comes to the efficiency of such studies. After the population reaches the carrying capacity of the environment, the growth rate slows down and so does the number of new mutations driving the evolutionary process. In our project, we solve this issue by using a morbidostat(1,2) for tracking the changes in growth rates in bacteria with slightly different sequences in a gene responsible for antibiotic resistance. This can give insights about the adaptive paths that populations take on fitness landscapes during evolution.

Figure 1: Experimental design

Experimental Design

We inserted altered plasmids in different bacterial cultures. All plasmids contained a mutated chloramphenicol resistance gene. We hypothesized that mutations in the chloramphenicol resistance plasmid would cause changes in bacterial fitness in an environment containing the agent. To observe this adaptive process in the short time frame of the SMTB, we grew the cultures in a morbidostat.

We took a sample of each population at the same time on two consecutive days, the second and third day of the experiment. The genetic material was amplified and sequenced. After alignment, the sample sequences were compared to the original sequence in order to determine the number, type and position of mutations. We defined fitness as the ability to thrive in the presence of chloramphenicol. We measured fitness values by growing mutants from different populations in media with different amounts of antibiotic. We consider populations more adapted if they are able to survive at higher concentrations of the antibiotic.

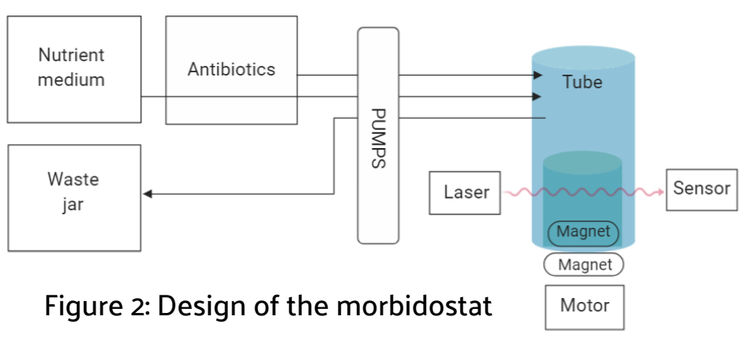

Figure 2: Design of the morbidostat

The morbidostat enabled the regulation of the antibiotic concentration in the nutrient media based on the bacterial growth. Antibiotics and nutrient medium were periodically being pumped into the tubes containing the bacterial culture, while excess liquid was being vacuumed into a waste jar. Magnets were placed inside and under the tubes, the latter of which were attached to spinning motors in order to keep the bacteria equally distributed along the tubes. Lasers and sensors monitored the bacterial growth by measuring the optical density (OD). When the OD reached a predefined threshold, the Raspberry Pi used to control the morbidostat would enter a loop and dilute the culture. The machine can be controlled remotely with a Telegram bot.

References:
1) Toprak E, Veres A, Yildiz S, et al. Building a morbidostat: an automated continuous-culture device for studying bacterial drug resistance under dynamically sustained drug inhibition. Nat Protoc. 2013;8(3):555–567. doi:10.1038/nprot.nprot.2013.021
2)Toprak E, Veres A, Michel JB, Chait R, Hartl DL, Kishony R. Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. Nat Genet. 2011;44(1):101–105. Published 2011 Dec 18. doi:10.1038/ng.1034

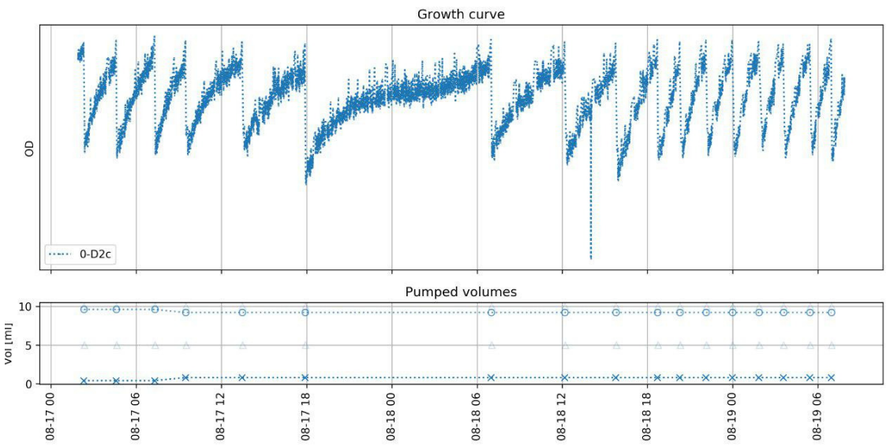

Figure 3: Culture density over time

Results

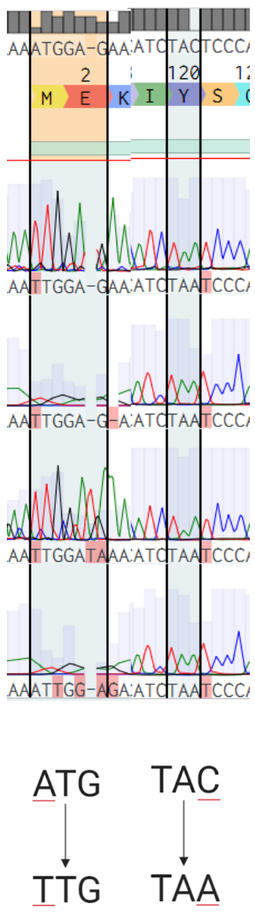

Figure 4: Sequencing results

In Figure 3, it can be seen that bacterial growth slows down after increasing chloramphenicol concentration. After acquiring the adapting to this concentration the growth accalerates.

The sequencing data showed changes only in one of 18 population. Initially the plasmid had a mutation in the start codon. After one day, a new start codon formed close by downstream. A stop codon, present in the initial mutant, was not repaired.

During the first day, bacteria did not demonstrate resistance to the antibiotic (orange and blue lines)., Increased OD, which we interpret as fitness, can be seen on the second day (red and green lines). However, higher densities 12 ug/mL than at 0 ug/mL chloramphenicol indicate technical errors.

Conclusion

Adaptation was seen in all bacterial populations, yet only one population had mutations in the chloramphenicol resistance plasmid. This might have happened because in other populations mutations occurred outside the plasmid of interest, e.g. in the chromosomal DNA of the bacteria. Alternatively, changes in only few of many copies of the plasmid per cell might have caused the fitness change. Results gotten from the fitness assay can also be questioned. Higher density was observed in antibiotic-rich wells in some of the populations compared to wells with less or without antibiotic (see Figure 5). This could have been caused by different ratios of dead and living cells at the time of inoculation.

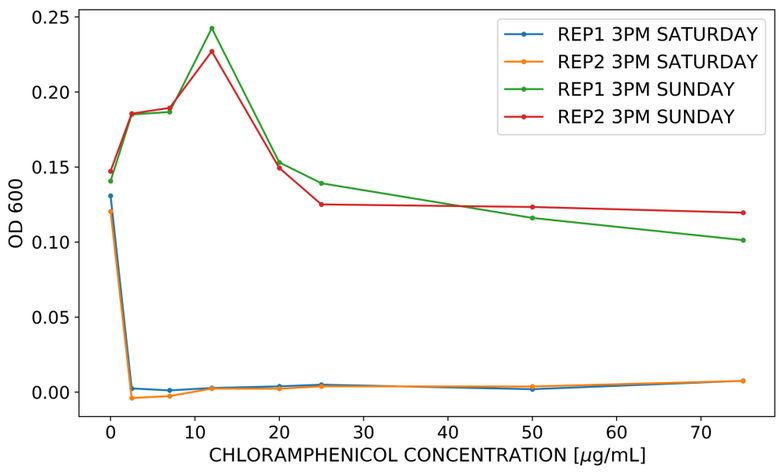

Figure 5: Fitness assay