Determining the relative fitness of *E. coli* populations evolved in carbon-limited or nitrogen-limited media in the presence of ampicillin

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Introduction

The successful treatment of many bacterial infections can be attributed to antibiotics. However, as these therapeutics become increasingly accessible, the use of antibiotics has led to an elevated percentage of antibiotic resistant bacteria—bacteria that evade engineered inhibitory mechanisms, making established treatments less effective.[1] One species of particular significance to the study of antibiotic resistance is *Escherichia coli*, having been identified by the World Health Organization as a critical priority due to its multi-drug resistance potential.[1]

Background

In Summer 2022, we designed and executed a project in which 36 *E. coli* populations were evolved in either carbon or nitrogen-limited media under a specific mode of ampicillin antibiotic treatment (**Fig. 1**). Each day, the bacterial cultures were transferred to a fresh flask containing the prescribed media and antibiotic treatment before being incubated again. Four of the ramping populations evolved under nitrogen limitation went extinct over the evolution period. After 16 days, a freezer stock was prepared for each of the remaining evolved *E. coli* populations for use in later projects.

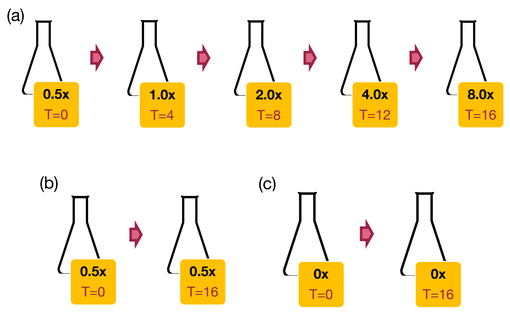

Figure 1. Antibiotic treatments used in the evolution experiment: (a) ramping, (b) constant, (c) blank

Objective

The purpose of this procedure was to discern differences in fitness between the populations which were evolved over the 16-day period and ancestral strains of *E. coli* via execution of a fitness competition. It was expected that the populations derived from the evolution experiment have elevated fitness relative to the ancestor.

Methods

In each fitness competition, an evolved population and an ancestor (REL 606 or REL 607) were inoculated into the same flask. To replicate the evolutionary conditions, the media was either carbon (DM25) or nitrogen-limited (DM250) with an antibiotic treatment corresponding to the evolutionary environment. The contents of the flasks were plated both on day zero and on the final day of the competition. For populations evolved in the absence of antibiotic, the fitness competition was extended over three days in order to better discern fitness differences (**Fig. 2**). Populations under ramping or constant conditions participated in competitions lasting one day (**Fig. 3**).

Populations derived from REL 606 appear dark red when grown on tetrazolium arabinose (TA) plates while populations derived from REL 607 appear white when grown on TA plates (**Fig. 4**). Therefore, the number of ancestral and evolved colonies present on each TA plate was determined on basis of color. From the number of colonies present on each plate, the relative fitness was calculated via application of the Malthusian parameter—the growth rate associated with a portion of a total population.[2]

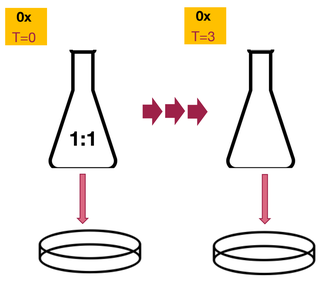

Figure 2. Overview of competitions completed in the absence of ampicillin with the ratio of evolved to ancestor listed on the T=0 flask

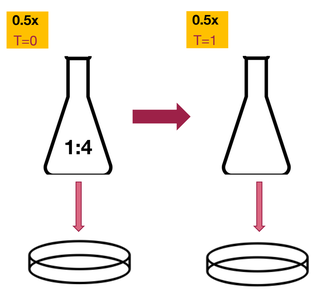

Figure 3. Overview of competitions completed in the ramping and constant evolutionary environments with the ratio of evolved to ancestor listed on the T=0 flask

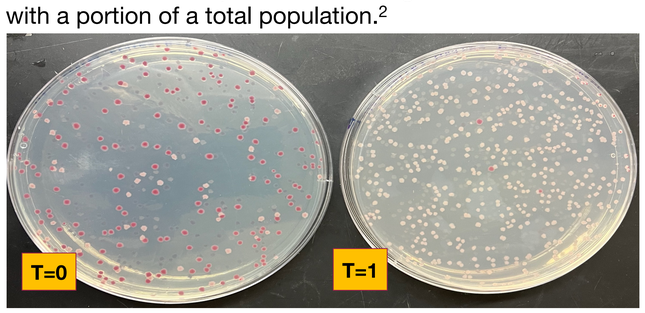

Figure 4. Results of fitness competition between population 3 (white) and ancestor (red) in DM25, ramping conditions

Sources:
(1) Mancuso, G.; Midiri, A.; Gerace, E.; Biondo, C. Bacterial Antibiotic Resistance: The Most Critical Pathogens. *Pathogens* **2021**, 10, 1310.
(2) Murray, Bertram G. Population Dynamics, Genetic Change, and the Measurement of Fitness. *Oikos* **1990**, 59, 189–99.

Results

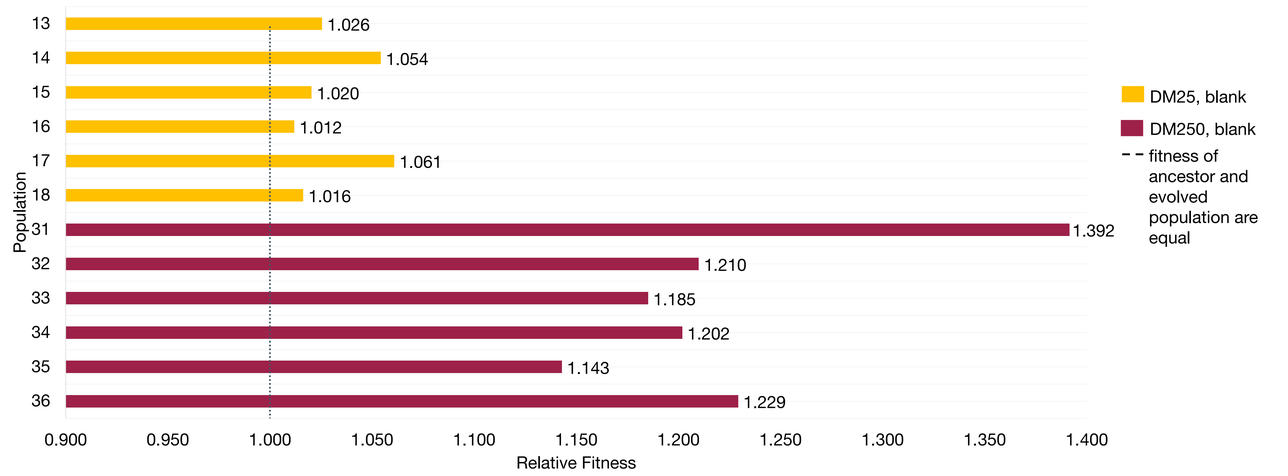

Figure 5. Relative fitness values of populations not treated with ampicillin. Populations evolved in DM250 experienced elevated fitness compared to those evolved in DM25

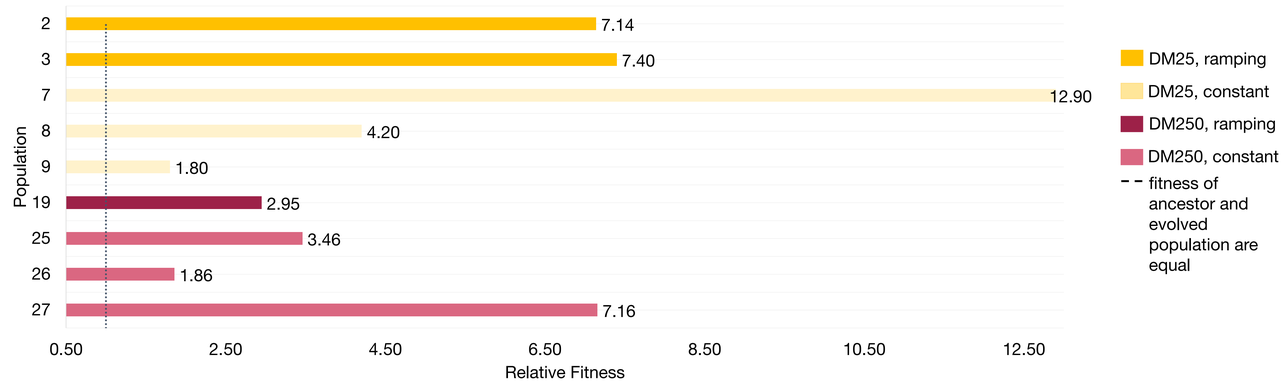

Figure 6. Relative fitness values of populations under constant or ramping ampicillin treatment

Discussion

As anticipated, each evolved population was more fit relative to the ancestor which it competed against. Interestingly, it appears that the populations which were evolved under nitrogen-limitation without ampicillin experienced a greater difference in fitness between ancestor and evolved than those populations which were evolved under carbon-limitation (**Fig. 5**). *This trend suggests that there was more opportunity for rapid fitness gains in the nitrogen-limited environment than in the carbon-limited environment in the absence of ampicillin*.

Regarding the populations which were evolved in either carbon or nitrogen-limitation under ampicillin treatment, no obvious trend presents itself from the relative fitness data. These populations experienced substantial differences in fitness between ancestor and evolved as would be expected under the strong selection pressure of ampicillin (**Fig. 6**). However, with the current data available, no trend can be applied to these populations.

It is important to note that, at present, some of the populations evolved under antibiotic conditions in the evolution experiment have yet to be used in a fitness competition. The remaining populations will follow the preceding procedure to determine their relative fitness values. It is expected that these fitness values will be high relative to the fitness values of the populations evolved in the absence of ampicillin.

Additionally, the lack of trend observed in **Fig. 6** may be the result of <10 ancestral colonies present on the T=1 TA plates. The small number of colonies causes the Malthusian parameter to be inherently noisy. In the future, the experimental conditions should be revised such that more ancestral colonies are present on the T=1 TA plates. This may be resolved by adjusting the antibiotic concentration or adjusting the ratio of ancestor to evolved in the competition flask.

Conclusion

- Each population evolved in the evolution experiment was more fit than the ancestor which it competed against
- Nitrogen-limited media appears to be more conducive for evolution of *E. coli* in the absence of ampicillin
- The remaining fitness competitions need to be executed; at that time, trends regarding the ramping and constant populations may be more discernable