Stress Introduction Rate Impacts Acquisition of Antibiotic Tolerance and Resistance

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***Short Abstract* — Stress tolerance studies are typically conducted in an all-or-none fashion. However, in realistic settings, cells may encounter stresses, including antibiotics, at different rates. Thus, how cells tolerate stress may depend on its rate of appearance. To address this, we study how the rate of introduction affects tolerance using a key stress response mechanism, efflux pumps. We found that slower rates of introduction provide a disproportionate benefit to efflux pump-containing strains, allowing cells to survive beyond their original inhibitory concentrations. We hypothesize cells surviving these stressful environments for longer periods will be predisposed to acquire mutations for antibiotic resistance.**

***Keywords* — dynamic environments, stress response, *E. coli*, antibiotics, efflux pumps**

I. Background

ANTIBIOTIC resistance has become a major public health concern as bacteria produce strategies to evade drugs, leading to recurring infections and overuse of antibiotics. Understanding the complex strategies bacteria employ to resist treatment could provide insight into different methods of targeting these evasive cells. Recent literature suggests that antibiotic resistance may be achieved after cells have gained tolerance in particular environments [1].

As a case study, we use efflux pumps to investigate how antibiotic resistance may be more likely to occur based on the environmental dynamics. Efflux pumps, such as AcrAB-TolC from *Escherichia coli*, are membrane transporters well known for their ability to export a wide variety of substrates, including antibiotics and biofuels [2-3]. As such, efflux pumps may be able to maintain a low intracellular concentration in stressful environments; and by doing so, enable long term survival and perhaps mutations.

II. Results

Our goal is to quantitatively determine the impact of short-term and long-term environmental dynamics on bacterial populations. To achieve this we co-culture cells with and without the efflux pumps. The relative fraction of cells with and without the pumps can change with time under variable antibiotic conditions.

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A. Short-term dynamic environments

We hypothesized that the ideal pump expression level would involve a rate-dependent trade-off between the benefit of pumps and the cost of their expression. To test this, we evaluated the benefit of the AcrAB-TolC pump under different rates of stress introduction, including a step, fast ramp, and gradual ramp using the antibiotic chloramphenicol. A mathematical model describing these effects predicted the benefit as a function of the rate of stress introduction. [4]

B. Long-term dynamic environments

Next, we hypothesized that long-term environmental perturbations enabling survival could produce permanent changes through mutations. Using a modular turbidostat, the eVOLVER, we were able to apply long-term dynamics to populations of bacteria [5]. We then quantify the population changes over several days to a week and check when mutations began to emerge.

III. Conclusion

Thus far, we have demonstrated that the benefit of efflux pumps depends heavily on the rate of stress introduction. We found that strains exposed to slower stress introduction rates were able to survive cumulative concentrations well beyond what they could survive if the stress appeared suddenly. This led us to investigate the long-term consequences of this increased tolerance through acquired resistance. Overall, this work will provide insight into how bacteria optimize the use of efflux pumps as strategic mechanisms for surviving and mutating in stressful conditions.

References

[1] Levin-Reisman C, et al. (2017) Antibiotic tolerance facilitates the evolution of resistance. *Science* 355:826-830.

[2] Elkins CA, Nikaido H (2002) Substrate Specificity of the RND-Type Multidrug Efflux Pumps AcrB and AcrD of *Escherichia coli* Is Determined Predominately by Two Large Periplasmic Loops. *J Bacteriol* 184:6490-6498.

[3] Sun J, Deng Z, Yan A (2014) Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochemical and Biophysical Research Communications* 453:254-267.

[4] Langevin AM, Dunlop MJ (2018) Stress introduction rate alters benefit of AcrAB-TolC efflux pumps. *J Bacteriol* 200:e00525-17.

[5] Wong BG, Mancuso CP, et al. (2018) A generalizable experimental framework for automated cell growth and laboratory evaluation. *Nature Biotechnology*, in press.