Established microbial colonies can survive Type VI secretion assault

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***Short Abstract* — Type VI secretion (T6S) is a cell-to-cell injection system that can be used as a weapon and is present in ~ 25% of sequenced Gram-negative bacteria. To examine the ecological role of T6S, we competed self-immune T6S+ cells and T6S-sensitive cells in simulated range expansions. As killing takes place only at the interface between sensitive and T6S+ strains, while growth takes place everywhere, sufficiently large domains of sensitive cells can achieve net growth in the face of attack. We validated these findings through *in vivo* competition experiments between T6S+ *Vibrio cholerae* and T6S-sensitive *Escherichia coli*. We found that *E. coli* can survive and even dominate so long as they have an adequate opportunity to form microcolonies. Finally, in simulated competitions between two equivalent T6S+ strains, the more numerous strain has an advantage that increases with the T6S attack rate.**

***Keywords* — Type VI secretion, microbial competition, evolutionary dynamics, simulation, agent-based modeling**

I. Introduction

Microbes employ a staggering range of extracellular tools to engineer their immediate environment [1]. Very often that environment is defined by the multitude of other cells in close proximity. The Type VI secretion system (T6SS) is a mechanism for direct cell-to-cell manipulation of these neighbors through the translocation of effector proteins [2]. By far the most commonly observed function of T6S is attack [4]. Specialized T6SSs can directly damage both prokaryotic and eukaryotic target cells through the translocation of toxic proteins directly across the membrane. T6SSs are present in approximately 25% of the Gram-negative genomes studied by Boyer and colleagues [3]. Antibacterial T6SSs appear to be found with cognate immunity proteins in every case [4]. Given this tactical advantage, one might expect T6SS to be even more widespread. Why is T6S not universal?

II. Methods and Results

To address the question of T6S's utility, we focused on the case of cell-to-cell killing between bacteria. We explored this scenario through the use of individual-based models (IBMs). We first developed an IBM that competes self-immune T6S+ and sensitive individuals in a range expansion, analogous to a surface colony (2D) or a biofilm (3D). We find that cell growth from the inside of an established domain can offset cell death at the interface between a T6S-sensitive strain and a self-immune T6S attacker. Consequently, given a sufficiently large domain, T6S-sensitive strains can survive T6S attack. The sensitive strain does not require a growth advantage to survive. Given even a small growth advantage, the T6S-sensitive strain can outcompete a self-immune T6S+ competitor.

We validated these findings through *in vivo* competition experiments between T6S+ *V. cholerae* and T6S-sensitive *E. coli* (Fig. 1). In these 2D plate assays, *E. coli* can form persistent microcolonies that survive, provided the initial local density of *V. cholerae* is not too high. Along similar lines, simulated competitions between self-immune T6S+ strains reveal that the initially more numerous strain benefits most from higher attack rates.

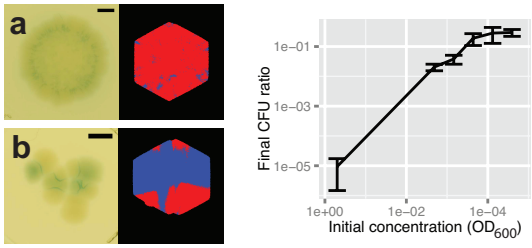

Fig. 1 Domain size predicts T6S-sensitive survival. Comparisons of experimental to simulation outcomes. (a) Left, overnight growth on x-gal media from an inoculum consisting of *V. cholerae* str. 2740-80 (LacZ-) and *E. coli* MG1655 (LacZ+), starting from equal amounts of OD600 = $2x10^{-4}$ culture from each species. Right, simulated range expansion from 729 T6S+ individuals and an equal number of sensitive individuals. (b) 9-fold dilution of simulated and experimental conditions, showing increased survival of sensitive *E. coli*. (c) Final ratio of sensitive *E. coli* to T6S+ *V. cholerae* as a function of initial inoculum concentration.

Acknowledgements: This work was supported in part by National Science Foundation Grants PHY-1305525, MCB-1119232, and MCB1344191.

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