Nutrient Gradients Mediate Complex Colony-Level Antibiotic Responses in Structured Microbial Populations

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***Abstract*— In structured bacterial populations, the coupling between bacterial metabolism and environmental transport processes, such as metabolite diffusion, creates large-scale nutrient gradients, which can affect cell growth, gene expression and drug susceptibility in different parts of the population. How resistance expression is coordinated in the presence of such spatiotemporal environmental coupling remains elusive. Using a custom microfluidic device [1], we observe the response of tetracycline-resistant *E. coli* microcolonies to precisely defined dynamic drug regimens. We show the formation of a resistant layer in the colony interior that can be quickly activated upon future drug exposures [2]. A mathematical model linking metabolism and gene expression regulation is able to capture the main features of spatiotemporal colony dynamics.**

I. Introduction

To explain the large-scale reorganization of microbial populations in variable environments, we need a quantitative understanding of how environmental cues are differently sensed and processed by single cells and integrated into complex behaviors at the community level. Here, we investigate how phenotypic diversity originating from environmental variations increases resistance at the population level during drug responses.

II. Results

We focus our analysis on the tet operon, which provides resistance against tetracycline (a translation inhibitor) in *E. coli*. We built a microfluidic device that allows fast switching between different media, which permits sudden exposure of the colony to a high dose of drug. Each trap housing a microcolony is connected to a nutrient supply channel that continuously delivers fresh medium to one side of the growing colony. As a result, cells closer to this side grow fast, while cells toward the interior of the microcolony grow less.

A. Transient Growth in Colony Interior Increases Resistance for Subsequent Drug Exposures

A sudden exposure to tetracycline arrests the growth of previously fast-growing cells at the edge of the colony, which increases the availability of nutrients to cells in the colony interior. Dormant cells in the interior are then temporarily reactivated, repopulating the edge of the colony and expressing high levels of resistance, until growth at the edge is resumed. Again depleted of nutrients, cells in the interior of the colony return to dormancy, retaining high levels of the resistance proteins expressed during transient growth. Subsequent exposures lead to further build-up of resistance proteins in these cells.

B. A Model Linking Metabolism and Gene Expression Captures the Main Features of Spatiotemporal Colony Dynamics

We developed an agent-based computational model of bacterial growth incorporating the coupling between direct resistance regulation, metabolism and environmental interactions. Each cell in the simulation is equipped with a gene regulatory circuit for the expression of resistance genes [3], where growth and expression are sensitive to both drug concentration and nutrient availability, and nutrient concentration in the environment is tracked separately by a continuous field throughout the trap. Our model was able to recapitulate the qualitative dynamics of spatially heterogeneous resistance expression and growth pattern modulation - most notably the reactivation of dormant cells upon drug exposure and the persistent accumulation of resistance in dormant cells.

III. Conclusion

Structured microbial colonies can maintain cell growth during drastic environmental changes that would easily arrest a planktonic population, utilizing a colony-wide mechanism of resistance where transient growth promotes high expression of resistance in dormant cells in the interior. Uncovering the fundamental principles that govern collective mechanisms of antibiotic resistance in spatially extended populations will allow the design of optimal drug regimens to counteract them.

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