The bacterial population's spatial structure non-monotonically impacts bacterial growth

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***Short Abstract* — In most natural settings, most bacteria are found in spatially-structured, pluralistic communities. The spatial structure of these communities governs interactions within and between species, and with the environment. Here, we present two cases in which cooperative and competitive inter-bacterial interactions impact growth fitness. (1) The per-cell likelihood of growth for antibiotic-resistant mutants in an antibiotic environment depends non-monotonically on the density of the surrounding, antibiotic-susceptible wild-type cells. (2) The relative growth fitness of large, multicellular aggregates, compared with single cells, depends on the density of competition, which is set by the concentration of single cells.**

***Keywords* — *Pseudomonas aeruginosa*, cooperation, competition, antibiotic, aminoglycoside, spatial structure, growth substrate, modeling, Poisson distribution.**

I. Spatial structure in bacterial populations

Most microbial communities consist of interacting, multispecies populations with inter- and intra-species interactions governed by the spatial structure of the microbial population and the environment. Recent work has shown that heterogeneity in the spatial distribution of antibiotic in the environment can accelerate the evolution of genetically-based antibiotic resistance [1,2]. Here, we examine the impact of ecological changes resulting from the spatial distribution of the microbial population on the growth of *genotypic* antibiotic resistance.

Biofilms are three-dimensional, sessile communities that promote *phenotypic* antibiotic resistance and differentiated patterns of gene expression and growth [3]. Differentiation is often linked to the positioning of cells in the biofilm structure, which helps control resource transport. Three-dimensional, multicellular aggregates can slough off to seed new biofilms, yet their role in seeding new biofilms is unknown.

Here, we examine how the spatial structure of the microbial population impacts growth fitness as the result of ecological interactions of bacteria with their environment. Our primary model organism is *Pseudomonas aeruginosa*, an opportunistic human pathogen that notoriously forms biofilm infections.

II. Growth of antibiotic-resistant mutants

In the presence of aminoglycoside antibiotic, we find that the per-cell likelihood of growth for antibiotic-resistant mutants depends non-monotonically on the overall cell density, which is primarily antibiotic-susceptible, wild-type (WT) bacteria. Two effects compete: mutants are inhibited by an alkaline, diffusible catabolic by-product, and protected when the local concentration of WT cells is sufficiently high to reduce the per-cell concentration of antibiotic+inhibitory factor below an effective threshold. We use the Poisson distribution to describe local fluctuations in cell density as a function of overall cell density and show that the resulting model describes our experimental data well [4].

III. Relative fitness of biofilm structures

When the overall cell density, and therefore the competition for growth resources, is low, we experimentally find that single cells have a growth advantage over multicellular aggregates. However, when competition is high, multicellular aggregates have a growth advantage over single cells. Agent-based modeling shows that cells in the aggregate interior have restricted access to growth substrate and therefore produce fewer progeny than do exterior cells. When competition is low, single cells have unfettered access to growth substrate and therefore have an overall growth advantage over the aggregate. However, when competition is high, the height of multicellular aggregates gives cells at the top better access to growth substrate, so that aggregates are at an overall growth advantage [5].

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