

# THE EFFECT OF MICROBIAL SPATIAL SELF-ORGANIZATION ON TRANSFER OF ANTIBIOTIC RESISTANCE GENES

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## Abstract

Range expansion is a general feature of surface-attached microbes. It involves the non-random assortment of microbes across space. This process is referred as spatial self-organization (SSO), which is a consequence of microbial behavior. SSO can generate spatial patterns that affect both ecological and functional aspects of microbial communities. Importantly, different spatial patterns can result in various magnitudes of intermixing, which further results in different frequencies of cell-to-cell contacts and different local population sizes. I hypothesize that SSO is an important determinant of plasmid-mediated horizontal gene transfer (HGT) and proliferation of antibiotic resistance genes (ARGs) within microbial communities. I predict that spatial patterns with greater intermixing between genotypes will promote HGT of ARGs due to more direct cell-cell contacts.

## Introduction

The spatial patterns with greater intermixing will result in smaller local population sizes, and consequently allow transconjugants to proliferate and establish themselves more easily. In order to test these predictions, I performed microbial range expansion experiments with a synthetic microbial community consisting of two genotypes of microbes. The uniqueness of this system is that I can obtain different spatial patterns via modifying the mode of metabolic interaction between the two genotypes. In my presentation, I will demonstrate the conceptual basis of my research questions and hypotheses. I will also outline my experimental system and methodological approach used to address my questions, including the synthetic model system and techniques to visualise and quantify transfer event. Ultimately, I hope to understand the relationship between microbial SSO and transfer of ARGs, which is of critical relevance to some of the most pressing problems facing our society, such as the transmission and spread of antibiotic-resistant genes during microbial range expansion.

## Material and methods

### (a) Experimental model system

We have described all of the strains used in this study in detail elsewhere [22,54,55]. For this study, our experimental model system is composed of four isogenic mutant strains of the bacterium *Pseudomonas stutzeri* A1501 (figure 2; electronic supplementary material, table S1). Briefly, the two complete reducers do not carry any disruptions in the denitrification pathway and can completely reduce nitrate (NO<sub>3</sub><sup>-</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>), nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and finally to dinitrogen gas (N<sub>2</sub>) (figure 2a; electronic supplementary material, table S1). By contrast, the producer carries a single loss-of-function deletion in the *nirS* gene and can reduce nitrate but not nitrite.

### (b) Range expansion experiments

We performed anaerobic range expansion experiments as described in detail elsewhere [22,25], which is a modified version of a protocol developed for aerobic range expansions [12]. Briefly, we first grew the two complete reducers, producer and consumer independently in aerobic lysogeny broth (LB) medium overnight in a shaking incubator at 37°C at 220 rpm. After reaching stationary phase, we adjusted the densities of each culture to an optical density at 600 nm of one (OD<sub>600</sub>), centrifuged the cultures at 3600g for 8 min at room temperature, discarded the supernatants and suspended the cells in 1000 µl of 0.9% (w/v) saline solution. We then transferred the cultures into a glove box (Coy Laboratory Products, Grass Lake, MI, USA) containing a nitrogen (N<sub>2</sub>) : hydrogen (H<sub>2</sub>) (97 : 3) anaerobic atmosphere, mixed the two complete reducers together or the producer and consumer together at a volumetric ratio of 1 : 1 and deposited 1 µl of each mixture onto the centre of a separate anaerobic LB agar plate amended with 1 mM of sodium nitrate (NaNO<sub>3</sub>) and adjusted to pH 7.5 with 0.5 M NaOH.

To assess the effects of physical objects on interspecific mixing, diversity and spatial self-organization, we used Nafion particles with a size range of 35–60 µm (Sigma-Aldrich, Buchs, Switzerland). Briefly, after we inoculated the anaerobic LB agar plates with pairs of complete reducers or pairs of producer and consumer, we incubated the plates for 48 h. This allowed for the suspension liquid from the inoculum to dissipate and for individual cells to attach to the LB agar surface. After 48 h, we then deposited approximately 5 mg of dry Nafion particles (i.e. the particles were not suspended in solution) to the inoculation area of each plate that was assigned to the physical object

treatment group and continued to incubate the plates for a total of two weeks. We note here that the growth-limiting substrate is nitrate (NO<sub>3</sub><sup>-</sup>) for all of our experiments, which we exogenously added to the LB agar that resides below the Nafion particles. We therefore do not expect the Nafion particles, which reside on the top of the LB agar, to constrain the diffusional supply of nitrate.

### (c) Microscopy and image analysis

We imaged the range expansions with a Leica TCS SP5 II confocal microscope (Leica Microsystems, Wetzlar, Germany) as described in detail elsewhere [22,25]. Briefly, prior to imaging the range expansions, we exposed the LB agar plates to ambient air for 1 h to induce maturation of the fluorescent proteins [22,25]. We then quantified the circularity of the range expansion area using the circularity isoperimetric quotient ( $\text{perimeter}^2/4 \times \pi \times \text{area}$ ) [53]. We next quantified the number of interspecific boundaries between two complete reducers or between the producer and consumer by first plotting a line at 50 pixels from the leading edge of the range expansion area.

## 3. Results

We first tested whether the addition of physical objects to a surface creates immediate deformities along an expansion frontier. To accomplish this, we assembled pairs of complete reducers together (1 : 1 initial cell number ratio) and deposited them onto separate replicated LB agar plates (n = 18). We then added Nafion particles as physical objects to half of the plates, immediately interrogated the expansion frontier via microscopy and quantified the effects of physical objects on the shape of the expansion frontier.

## 4. Discussion

We show here that physical objects can have important effects on the interspecific mixing, diversity and spatial self-organization of microbial communities. Perhaps surprisingly, the effects of physical objects on the density of interspecific boundaries depend on the type of interaction that occurs between the resident cell types.