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The Ecology of Bacterial Individuality

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Summary

Classical microbial ecology generally describes and quantifies bacteria at a population or community level, be it for example in a liquid culture, as a colony on an agar plate, or as found in environmental samples. Such an approach necessarily yields results that reflect population or community averages, e.g. an average enzyme activity, an average doubling rate, or an average experience of the environment. The latter is at odds with the growing realization that considerable environmental heterogeneity exists in physicochemical and biological properties at the micrometer-scale. By averaging the bacterial experience of such heterogeneous environments, potentially valuable information is lost about the behavior of the organisms under study and about the variation in that behavior. Recent technical advances have introduced new possibilities to interrogate individual bacteria for their perception of and response to their local environment.

In this thesis, we used the phyllosphere, or leaf surface, as a model bacterial environment to address the question if and how individual cells that colonize this habitat are impacted by micro-scale environmental heterogeneity and how these impacts help to shape bacterial populations. To this end, a novel bioreporter was introduced that enabled analysis of single-cell reproductive success of individual bacteria. This new bioreporter was termed CUSPER (reproductive success = repsuc read backward) and is based on the dilution of green fluorescent protein (GFP) from growing cells of the natural leaf-colonizing bacterial strain *Erwinia herbicola* 299R.

By applying CUSPER cells to bean leaf surfaces, it was revealed that individual immigrants differed in their ability to produce offspring and contributed differently to the final observed populations. This was interpreted as an imposed effect of the environmental heterogeneity on the immigrating population, suggesting that the phyllosphere represents a patchy environment that offers various degrees of habitability to members of an immigrant population.

Subsequently, CUSPER was used to quantify the impact of different leaf inoculation densities on the reproductive success of cells within a population of bacterial immigrants. Some of the chosen densities were deliberately in excess of the carrying capacity of the bean leaves under study. This approach made it possible to show that even under circumstances in which immigrants arrive at numbers higher than the leaf can sustain, some cells were still able to reproduce. This finding suggested that carrying capacity has to be understood as the sum of many local carrying capacities. A complementary modeling approach revealed that experimental observations can be explained by the existence of three types of microenvironments. Two of these environments represented approximately equal parts of the bean leaf surfaces under

study and offered low or intermediate reproductive success. A third class of micro-environment made up only 2% of the environment and offered high reproductive success.

A subsequent study dealt with the effect of pre-colonization of leaves on individual secondary colonizers. This is highly relevant in the context of preemptive colonization, a biocontrol approach that is often used to prevent the growth of plant-pathogenic bacteria. The study revealed that, even at high densities of precolonization, individual secondary colonizers were able to divide often enough to create as many as eight offspring, while the corresponding total population showed no net increase. Much of the heterogeneity of the leaf surface is due to local differences in nutrients that leak from the leaf interior to the surface. To obtain estimates for the rate at which this process takes place at the micrometer scale, we used an integrated approach of fructose-bioreporting bacteria and gas-chromatography, coupled to flame ionization detection, to determine the local permeability of isolated poplar leaf cuticles for carbohydrates. The average permeability of the poplar cuticles for fructose was estimated to be $3.39 \times 10^9 \text{ m s}^{-1}$. Microscopic analysis of cuticles that were inoculated with bioreporting bacteria showed a non-random distribution of fructose-reporting bacteria. By relating the area covered by fructose-reporting bacteria with the measured average permeability, it could be shown that the permeability of poplar cuticles is locally 270 times higher than the rest of the cuticle. These findings gave an indication that resource partitioning might play an important role in the explanation of different reproductive success rates of bacterial immigrants to the phyllosphere.

In an attempt to combine the findings and models established in the previous parts of the thesis, a spatially explicit, agent-based modeling approach for bacterial colonization of the phyllosphere was formulated. The model, coined ASiMoPh for Agent-based Simulation of Microbial Phyllosphere colonization, was able to simulate the patterns observed in the previous studies using a simple set of rules for reproduction and nutrient uptake of bacterial agents. The model consisted of a two dimensional world that offered immigrant agents three different classes of microenvironments featuring different qualities in initial resource availability and resource replenishment.

The final study in this thesis presents the preliminary results of the sequencing of the strain *Erwinia herbicola* 299R. With the availability of the genomic information of this model phyllosphere organism, several new approaches will be possible, for example expression studies and comparative analysis of the genomes and functions between other phyllosphere strains to address the question: “What makes a successful phyllosphere bacterium?”

Taken together, the studies presented in this thesis demonstrated that single-cell observations yielded important information of the heterogeneity that shapes bacterial populations in the phyllosphere that were thus far overlooked. The CUSPER bioreporter offers an excellent tool for future studies investigating bacterial individuality not only in the phyllosphere, but also for many other environments.

Important first steps were taken in this thesis to build a conceptual framework for bacterial individuality in natural environments that may serve as a foundation for future research in individual-based microbial ecology.