Session: Biofilm ecology and ecotoxicology

Selection for – and impact of – evolved genotypic variants in multispecies biofilms

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In nature, microorganisms live in complex interacting communities with spatial structure, such as biofilms, which facilitates local co-existence of different genotypes. Furthermore, social interactions between the community members can lead to emergent activities by modified gene expression in the communities. Here, we examined the effects of spatiotemporal dynamics on evolution of adapted variants over a short timespan to determine the link between interspecies interactions in biofilms and selection pressure for new phenotypes possibly impacting the structure and composition of the biofilm matrix.

Co-isolated environmental species *Bacillus thuringiensis, Pseudomonas laurentiana* and *Pseudomonas brenneri* were used for a short-term co-evolution experiment in both a static cultivation system allowing for biofilm development, and in a cultivation that favored planktonic growth, to examine the impact of selection pressure on coexistence in the spatial structure. Evolved strains were phenotypically characterized and sequenced to identify mutated genes and altered functions. Further, to better understand the selection pressure, the matrix composition was also characterized to examine if the presence of the novel variants affected it. The matrix characterization was performed both using a proteomic analysis of extracted samples and using a fluorescent lectin screening with 78 different lectins.

We show that new phenotypic variants of *B. thuringiensis* were found to be more abundant when grown in communities and they displayed altered spore development. The increased abundance of the new variants of *B. thuringiensis* when coexisting with the other species was found both when growing in the spatial structure and as planktonic growth. For one of the lectins, the wildtype *B. thuringiensis* strain showed a difference in binding for dual-species communities compared to mono-species communities which was not the case for the variants. The results so far reveal that interspecies interactions are able to directly affect extracellular traits of coexisting species in a biofilm. Furthermore, this study demonstrates that coexistence can influence the selection pressure of new variants able to impact the biofilm structure.