Permissiveness of soil microbial communities towards broad host range plasmids - DTU Orbit (09/01/2019)

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Horizontal transfer of mobile genetic elements facilitates adaptive and evolutionary processes in bacteria. Among the known mobile genetic elements, plasmids can confer their hosts with accessory adaptive traits, such as antibiotic or heavy metal resistances, or additional metabolic pathways. Plasmids are implicated in the rapid spread of antibiotic resistance and the emergence of multi-resistant pathogenic bacteria, making it crucial to be able to quantify, understand, and, ideally, control plasmid transfer in mixed microbial communities. The fate of plasmids in microbial communities and the extent of bacterial phyla permissive towards plasmid receipt are largely unknown. Historically, methods exploring the underlying genetic and environmental factors of plasmid transfer have been heavily reliant on cultivation and expression of plasmid encoded phenotypes. This has provided an incomplete and potentially cultivation biased image of the extent of plasmid transfer.

In this thesis, I investigated the extent of plasmid transfer in microbial communities at an unprecedented level of resolution and not reliant on cultivation. I focused on soil microbial communities. Their potential role as a reservoir for plasmids carrying antibiotic resistance genes is increasingly suspected to majorly contribute to the emergence of multi-resistant pathogens. More specifically, I examined what fraction of a soil microbial community is permissive to plasmids, identified the phylogenetic identity of this fraction and studied environmental factors that modulate plasmid transfer in soil microbial communities.

In order to attain these goals, I developed a high-throughput method that enabled me to evaluate the permissiveness of bacterial communities towards introduced plasmids. This new approach is based on the introduction of fluorescently tagged conjugative plasmids into a soil microbial community in solid-surface filter matings under maximized cell-to-cell contact, followed by quantification of transfer events through advanced fluorescent microscopy, isolation of transconjugants through triple-gated fluorescent activated cell sorting and finally 16S rRNA targeted pyrosequencing of the sorted transconjugal pools.

Employing this new method, I was able to map, for the first time, the diversity of all recipients in a soil microbial community for three broad host range model plasmids: RP4, pKJK5, and pIPO2tet. I found that a large fraction of soil the bacteria (up to 1 in 10,000) were able to take up any of these broad host range conjugal plasmids. The transconjugal pools comprised 11 bacterial phyla. This finding indicates that the realized transfer range of broad host range plasmids in environmental microbial communities is much larger than previously assumed. I was able to show abundant plasmid transfer from the Gram negative donor strains to a wide diversity of Gram positive soil bacteria, formerly thought to constitute distinct clusters of gene transfer. Moreover, among the observed transconjugants, I identified a core super-permissive fraction of taxa prone to receive diverse BHR plasmids from diverse donors. This fraction comprised the proteobacterial genera Pseudomonas, Enterobacterium and Burkholderia. These taxa are known to be evolutionary interlinked through chromosomal gene exchange. Hence, I was able to show that the gene pool of microbial communities may be directly interconnected through transfer of BHR plasmids at a so far unrecognized level.

The developed method furthermore enabled me to explore how agronomic practices may affect gene transfer in soil microbial communities. I compared bacterial communities extracted from plots subjected to different treatments for their permissiveness towards the model BHR plasmids RP4, pRO101 and pIPO2tet. Periodic manure introduction increased the permissiveness of the community towards these plasmids by up to 100% compared to control treatments. However, the phylogenetic composition of the transconjugal pools remained similar. The underlying mechanisms remain unclear. Subsequently, I focused on the effect of metal cations - Cu, Ni, Zn, and Cd – on community permissiveness. These cations are common environmental stressors associated with manure application to agricultural soils. I postulate an increased permissiveness of the community as a generic stress response to acquire foreign genes potentially conferring adaptive traits. I therefore evaluated to what extent short term metal stress modulated plasmid transfer. I analyzed both the transfer frequency and the phylogeny of the transconjugal pools using model BHR plasmid pKJK5 introduced through the y-proteobacterial donor E. coli. I found that the permissiveness towards plasmids was modified through stress on a taxon specific basis and cannot be generally predicted for the whole community. The response of the phylogenetic groups was specific for the metal and level of stress imposed. The phylum Bacteroidetes, for example, displayed an increased permissiveness at moderate (20% growth inhibition) but not at severe (50% growth inhibition) applied Cu or Ni stress. I therefore showed that specific metal stress can increase or decrease gene transfer between phylogenetically distant groups.

Finally, I extended the high-throughput method to quantify the potential of a microbial community to actively mobilize and transfer exogenous mobilizable plasmids to its indigenous members. I evaluated the transfer frequency of model plasmid RSF1010 by comparing it to the community's permissiveness towards the mobilizing, conjugal plasmid RP4 and to the rate of transfer between isogenic strains. My results indicated that retromobilization takes place at frequencies only one order magnitude lower than permissiveness for conjugal RP4 transfer. Mobilizable plasmids transferred in the communities at frequencies of up to 30 times higher than the conjugal plasmid RP4 itself when co-resident with a conjugal plasmid.

In conclusion, in this thesis I developed a novel toolbox to study plasmid transfer of conjugal and mobilizable plasmids in mixed microbial communities. This method allows, for the first time, a detailed mapping of the realized transfer range of plasmids. I discovered that a previously far underestimated fraction of bacteria in natural communities is directly interconnected through BHR plasmid transfer. While a super-permissive fraction of bacteria were able to take up plasmids at high frequencies from diverse donors, I showed plasmid or donor dependence of plasmid transfer to other species. Additionally, environmental factors like stress also impact the permissiveness of phylogenetic groups towards plasmids. The developed method and results increase our ability to predict the fate and impact of plasmids in microbial communities.