Permissiveness of soil microbial communities toward receipt of mobile genetic elements

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You are what you eat – how environmental signals foster horizontal gene transfer in *Vibrio cholerae*

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V. cholerae, the causative agent of the disease cholera, is primarily a member of aquatic habitats where it is often found associated with zooplankton and more specifically with their chitinous exoskeleton. Upon growth on such chitinous surfaces, *V. cholerae* initiates a developmental program termed 'natural competence for genetic transformation'. Natural competence for transformation is a mode of horizontal gene transfer in bacteria and contributes to the maintenance and evolution of bacterial genomes. In this study, we investigated the interconnection between three regulatory pathways involved in competence induction: sensing of chitin oligomers, carbon catabolite repression and quorum sensing.

We investigated diverse phenotypes of *V. cholerae*, which all contribute to chitin-induced natural competence. More specifically we used methods such as natural transformation assays, chitin surface colonization methods, transcriptional reporter fusions, quantitative RT-PCR and immunological detection of protein levels using western blot analysis. Furthermore, we created and studied a plethora of *V. cholerae* mutant strains.

We provide evidence that under homogeneous competence inducing conditions all members of a *V. cholerae* population become naturally competent. A more heterogeneous expression pattern is observable upon growth on chitin surfaces, most likely due to heterogeneity of environmental signals around the surface. Such environmental signals include chitin oligomers derived from the degradation of the chitin polymer, absence of preferred carbon sources, which would otherwise interfere with intracellular cAMP levels, and the abundance of species-specific autoinducers. We demonstrate that all these molecules are involved in competence induction in *V. cholerae*.

Our results illustrate the complexity of the regulatory network driving chitin-induced natural competence and transformation in *V. cholerae*. Most notably, we show that the fate of free DNA is mainly determined by quorum sensing: at low cell density surrounding DNA is degraded by extracellular nucleases. However, upon reaching a high cell density state, a switch from DNA degradation toward uptake of intact DNA strands occurs. This switch is based on the coregulation of a minority of chitin-induced competence gene by the quorum sensing pathway. We will conclude with a model of the regulatory circuit of chitin-induced natural competence in *V. cholerae*.

Hot spot of horizontal gene transfer: high abundance and diversity of mobile genetic elements in bacterial communities of on-farm pesticide bio-purification systems

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Plasmid mediated horizontal gene transfer (HGT) is recognized as a major force contributing to rapid adaptation of bacterial communities to changing environmental conditions. HGT processes can be triggered by various different stresses including various different compounds (for example antibiotics, metal, pesticides). Various human activities can increase frequencies of HGT in these hot spots. The enormously high densities, nutrient rich conditions and diversity of compounds foster HGT. While the mobilome of man-made hot spots such as sewage treatment plants or manure lagoons or tanks has been

extensively studied, no such in-sights were available for so-called on-farm pesticide bio-purification systems (BPS). BPS are rather simple, cheap and easy to maintain pollution control techniques using microorganisms to degrade pesticides. They are composed of straw, peat compost, soil, wood chips. BPS are being increasingly used on-farm to remove pesticide residues from water used for cleaning pesticide spraying devices. We hypothesized that plasmid mediated HGT might foster the adaptation of bacterial communities in BPS to the various pesticides used on farm and that these plasmids might carry genes involved in pesticide degradation. The mobilome of bacterial communities in BPS in Kortrijk, Belgium was monitored over the season in 2011 by means of cultivation-independent methods. Chemical analysis done for the same set of samples showed the presence of various pesticides in the ng to µg range per g of BPS (Flouroxypyr, Diflufencian, Bentazon, Metribuzin, Epoxiconazol, Terbuthylazin, S-Metalochlor, Flufenacet, Tebuconazol). For many of the pesticides detected concentrations peaked for the July sampling and later decreased. PCR amplification with primers targeting replicon sequences of different plasmid groups in combination with Southern blot hybridization revealed a remarkably high abundance of different plasmids belonging to the IncP-1, IncP-9, IncP-7, IncW, IncQ groups. Other plasmid groups such as IncU or IncN were less abundant. The diversity of plasmids detected is likely also to reflect bacterial community composition. Most remarkably all different IncP-1 groups recently reported from sewage treatment plants (Bahl et al., 2009) were also detected in BPS. In addition, integrase genes of class 1 and class 2 integrons were detected. To capture the intact plasmids and subsequently characterize their degradative genes, biparental exogenous isolations with different recipients and selective agents ($HgCl_2$, 2,4 D) were attempted. While a diversity of plasmids was isolated in P. putida the majority of them belonged to the IncP-9 group. Thus further adjustment of the isolation techniques is ongoing in order to access the impressive plasmid diversity present in BPS and to get hold of their degradative genes. Plasmid sequencing is in progress. In conclusion bacterial communities of BPS unlike soils have a much higher abundance of plasmids which provide likely a fitness advantage under the conditions of changing inputs of different pesticides which might serve as nutrient source when respective genes encoding for degradative enzymes are present.

Mapping genotypic diversity onto niche adaptation

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In bacteria, the fine-scale mapping between genotypic diversity and niche adaptation has remained elusive, mostly due to the labile nature of bacterial genomes and the microscopic scale of their interactions with the environment. Hence, determining the ecological implications of genotypic diversity in bacteria remains one of the most intriguing challenges in microbial ecology.

We used two closely related (99% nucleotide identity at the level of housekeeping genes) genotypic clusters of the *Vibrionaceae* that represent recently diverged ecological populations, to address fundamental questions about the link between fine-scale genotypic differences and niche differentiation. Despite their high genetic similarity, field studies show that these two populations differ in their microhabitat preference, with one population occurring primarily in large particles (>63 µm) and the other found on small particles (1-5 µm). We performed a detailed behavioral comparison of these two ‘ecotypes’ to understand how their ecological strategies differ. We find that these two populations partition in space, with the ‘large particle‘ group strongly attaching to surfaces relative to the ‘small-particle’ group. This difference in behavior corresponds exactly to the presence or absence of msh pili genes, which seem to have been recently acquired in the ‘large particle’ group. Because recent recombination has been depressed between these two groups, our results suggest that surface attachment has been the fundamental trigger of the ecological and genetic differentiation between these ecotypes.

The ecological dynamics of these marine bacteria is consistent with trade-offs between resource utilization and exploration, where 'large particle’ specialists, with large resource handling time, can exploit resource patches more efficiently than ‘small-particle’ individuals, which are better adapted at exploring new resource patches. Our data suggest a direct link between genetic mechanisms (msh genes) and

global ecological strategies and indicate that ecological theories of resource utilization trade-offs, classically used in plant and animal ecology to explain coexistence in non-equilibrium systems, also apply to bacteria in the ocean.

MetaGenomic Species: adding structure to metagenomics data

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A 3.9M microbial gene catalog derived from 396 human stool samples was binned into 7381 MetaGenomic Species (MGS) with high gene-wise abundance covariance. The MetaGenomic species range in size from 3 to 6319 genes, with 741 MetaGenomic Species containing 700 or more genes. MetaGenomic species are representative of biological units such as microbial organisms, plasmids, bacteriophages, genomic islands and more. The genes contained in the MetaGenomic Species show remarkable consistency in taxonomy, GC content and for more than 200 MetaGenomic Species a 'high quality draft genome' can be assembled. A large proportion of the assembled genomes show little or no similarity to any previously sequenced organism. The smaller MetaGenomic Species are enriched for genes characteristic for phages and plasmids, others show consistent functional annotation and some show strong dependencies to larger MetaGenomic Species. This is the first described unsupervised identification and nearly complete assembly of hundreds microbial genomes from a complex metagenomic sample series and the first global overview of the genetic interactions between bacteria, plasmids, phages and genetic islands in the human distal gut.

Insights into the bovine rumen plasmidome

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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. Plasmids often serve as mediators of lateral gene transfer, a process considered to be a strong and sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population in the environment of the bovine rumen, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep sequencing using the Illumina paired-end protocol and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and at various time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. The bacterial phylum distribution of the plasmidome was different from that of the rumen bacterial taxa. Nevertheless, both shared a dominance of the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Evidently, the rumen plasmidome is of a highly mosaic nature that can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen metagenomes, it became apparent that the rumen plasmidome codes for functions, which are enriched in the rumen ecological niche and could confer advantages to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

Tuesday 21 August
Contributed Session

Regulation of transfer of the ICEclc element of Pseudomonas
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Integrative and conjugative elements (or ICE) form an important new class of mobile DNA that largely contribute to bacterial genome evolution and adaptation. ICE are normally integrated in the host chromosome but can excise at low frequencies, upon which they transfer by conjugation to a new recipient cell and reintegrate. Here we study transfer of a mobile DNA named ICEclc of Pseudomonas knackmussii B13, an element that permits its host to use 3-chlorobenzoate as unique carbon and energy source.

By using single cell analysis we find that ICEclc becomes active and excises in only a few percent of all cells in a population during stationary phase conditions. We demonstrate that such cells are actual donor cells that transfer ICEclc. The main trigger for activation of ICEclc is noise in expression of the stationary phase sigma factor RpoS, which leads to a bistable activation cascade locking cells in a program determined by ICEclc. Quite astonishingly, active donor cells face severe growth defects compared to non-active donor cells when presented with new carbon substrate. Thus, ICEclc induces a cellular differentiation in donor cells to become transfer competent, which for those cells is a dead-end road.

Survival strategies of plasmids in chemostats and biofilms: effect of host-range and competition
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Some bacterial plasmids have a narrow (NHR) while others have a broad host-range (BHR). An extended host-range means more frequent swapping of hosts and therefore less time to adapt to the new host leading to a greater fitness burden on average. So what are the advantages of having a broad host-range? We hypothesized that BHR plasmids are more successful in multispecies communities than NHR plasmids, especially if the community is spatially structured. If this were true, BHR plasmids would facilitate the spread of antibiotic resistance genes through natural communities characterized by high diversity and spatial structure, for example the human gut.

To answer this hypothesis, we have developed mathematical models of the dynamics of transfer of BHR plasmids when competing with the faster growing NHR-plasmid bearing cells in multispecies communities. We compare a simple, deterministic mass-action model for the chemostat with a stochastic, individual-based model to evaluate the importance of random transfer and washout events. Further, we compare the well-mixed chemostat with spatially structured biofilms by using the same individual-based model as it can simulate both systems.

In the individual-based chemostat where each individual cell has the same chance of being washed out, we observe bottlenecks leading to failure to re-invade the community of hosts and the quick extinction of one or both plasmids. We also found that a costly NHR plasmid that can survive in a single species community cannot survive in a multispecies community. Curiously, adding a competing BHR plasmid helps the NHR plasmid to survive and coexistence of the two plasmids becomes possible under a range of conditions. In biofilms, the BHR strategy is the better competitor despite the higher costs. Increased interspecific competition between a specialist and a generalist plasmid seems to facilitate their long-term persistence.

We have also carried out filter mating experiments and growth curves in batch cultures with a range of lab strains of Escherichia coli,Pseudomonas putida andP. aeruginosain order to evaluate the effect of host background on plasmid transfer and fitness burden. We found a strong dependence of transfer frequency

and fitness burden of the plasmids on the host species or strain. Surprisingly, the majority of the lab strains tested either grew faster in presence of the plasmid or their growth rate was only marginally affected. Preliminary results of mating experiments on filters support the model predictions of a reduced plasmid transfer of the NHR plasmid in the presence of non-participating background species.

In conclusion, BHR plasmids appear to have advantages in multispecies chemostats and biofilms as originally hypothesized but the story is more complicated as the BHR plasmid can help the NHR plasmid to persist. Preliminary experiments so far support these findings but question the commonly made assumptions about fitness costs.

Permissiveness of soil microbial communities toward receipt of mobile genetic elements

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Horizontal gene transfer may provide microbial communities with adaptive capabilities to changing environmental conditions. The exchange of mobile genetic elements, such as plasmids, in soil microbial communities has been thoroughly documented. However, less is known about the actual fraction of a soil microbial community that is engaged in MGE dynamics. In addition, it is not known whether agronomic practices (soil treatments) might affect the ability of the community to accept MGEs. The aim of study was to determine for differently treated agricultural soils, the density and diversity of the soil microbial community members that receive exogenously added conjugal elements, termed community permissiveness.

Soil communities were isolated from untreated, manured - and Nitrogen/Phosphor/ Potassium treated soils at a test field site (Taastrup, Denmark) and from the TerraGenome soil (Park Grass, Rothamsted, United Kingdom). The broad-host range IncP-1 plasmids (RP4 and pRO101, a pJP4 derivate) and a cryptic plasmid (pIPO2tet) were tagged with an inducible green fluorescent reporter gene (*gfp*), and delivered in parent strain *P putida* KT2440, tagged with *lacI*^{q1} : *dsRed*.

Conjugation experiments were conducted as standardized solid-surface matings, where mixtures of indigenous soil bacterial suspension and donor cells were incubated on membrane filters laid on top of a 20% soil extract solid medium. Conjugation events were visualized by stereomicroscopy as the partly or completely GFP-expressing microcolonies on membrane filters, and transconjugant micro-colonies were retrieved by micro-manipulation for phylogenetic characterization. A broader characterization of plasmid host range for RP4 and pIPO2tet plasmids was performed by flow cytometric sorting followed by pyrosequencing of the transconjugant pool.

Large fractions of soil bacterial communities from treated soils were able to receive the tested conjugal plasmids. The phylogenetic identification of transconjugant micro-colonies revealed a very broad recipient range and significant differences between the investigated conjugal plasmids. Identified genera belonged to the *Alphaproteobacteria* (*Rhizobium, Bosea, Ochrobactrum* etcetera), *Betaproteobacteria* (*Burkholderia*, *Achromobacter* etcetera) and *Gammaproteobacteria* (*Stenotrophomonas*, *Citrobacter*, *Enterobacter* etcetera). In addition, several *Flavobacteria* and *Sphingobacteria* were retrieved as transconjugants. The results suggest a quick spreading and persistence of exogenously added conjugal elements to differently treated agricultural soils.
