Genefish: a window into targeted bacterial diversity

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CT17.001 EVIDENCE FOR EXTENSIVE LATERAL GENE TRANSFER IN ARCHAEAL AND BACTERIAL BIOFILM COMMUNITIES AT THE LOST CITY HYDROTHERMAL FIELD

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Densely populated microbial biofilms at the Lost City Hydrothermal Field are thriving in carbonate chimneys where fluids can reach 90°C and pH 10-11, conditions previously unknown in marine environments. The biofilms inhabiting the hottest, highest pH zones are dominated by a single archaeal phylotype (Lost City *Methanosarcinales*). The outer zones of carbonate chimneys, where hydrothermal fluids mix with cold, oxygenated seawater, are dominated by representatives of the *Thiomicrospira* genus. In this study, we present evidence of extensive lateral gene transfer (LGT) in both the archaeal and bacterial communities. Metagenomic sequencing identified a high abundance and diversity of sequences encoding transposases, the enzymes required for insertion of DNA during LGT. All previously reported metagenomic datasets contain at least an order of magnitude fewer transposases. Many of the transposase sequences were associated with large metagenomic contigs comprised of *Thiomicrospira*-like sequences, indicating that the *Thiomicrospira* pangenome is highly transposase-rich. Comparison of the *Thiomicrospira*-like contigs to the complete genome sequence of *Thiomicrospira crunogena* revealed substantial differences that must have arisen since the divergence of the two lineages, including large genomic rearrangements, gene fusion events, a prophage insertion, and transposase activity. The archaeal biofilm communities also show evidence of LGT. Targeted amplification and sequencing of the nifH nitrogen fixation gene from chimney biofilms revealed a wide range of sequences affiliated with methanogens even though only a single archaeal 16S rRNA phylotype was recovered from the same samples, suggesting that these nifH genes were acquired via LGT. These results suggest that rampant LGT among members of the Lost City archaeal and bacterial biofilm communities may serve as a generator of phenotypic diversity in communities with very low organismal diversity.

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CT17.002 GENOMIC ORIGINS OF SYMPATRIC SPECIES IN A SINGLE POPULATION OF *SULFOLOBUS ISLANDICUS*

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Sympatric divergence and ecological speciation in the presence of recombination has been shown in many macrobial species. With the ultimate goal of identifying the relative importance of homologous recombination (HR) and ecological selection in shaping the structure and speciation process within an archaeal population; we characterize the distribution, frequency, and genomic architecture of HR among the genomes of 12 strains of *S. islandicus* isolated from a single hot spring (pH=2, T=76°C) from the Kamchatka Peninsula. Phylogenetic and SNP patterns from full-genome alignments demonstrate the population is composed of two differentiated clusters plus several other rare recombinant genotypes. Although HR events occurred in all genomes, the intermediate lineages are largely the result of extensive recombination between members of the two differentiated clusters as well as the acquisition of fragments from transient relatives not present in the spring at sampling time. The distribution of HR events is uneven among strains, with a subset of two strains containing twice or more events than any other and having over a third of their genomes associated to HR. HR is more frequently found in sections we previously identified as variable regions, with the events displaying a wide variation in their size (0.024-79 Kb). We report differences in gene content that may indicate sources of ecological differentiation between divergent clusters and could be driving sympatric speciation in the population. Our results show that in a population of *S. islandicus* HR mediated the increase of genomic variation producing several recombinant lineages much like a "hybridization zone" between two differentiated ecotypes. The uneven HR distribution and signatures of potential evolutionary independence in recombinants suggest HR as capable to generate new lineages in an archaeal population and that the divergence of novel lineages is maintained despite the homogenizing force of recombination.

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CT17.003 *PSB*A DIVERSITY AND RECOMBINATION IN COASTAL CALIFORNIA CYANOPHAGE COMMUNITIES

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Cyanophages (viruses that infect cyanobacteria) are an abundant and diverse component of marine ecosystems that influence cyanobacterial community composition and nutrient cycling rates through host-specific mortality. Cyanophages are also thought to play a role in the evolution of their hosts through horizontal gene transfer (HGT). For example, many marine cyanophage carry a host-derived gene (*psb*A) that encodes a core photosynthetic protein (D1) and appears to increase viral fitness. Here we examine the diversity and evolution of cyanophage-encoded *psb*A from ~ 900 isolates from Southern California coastal seawaters. Cyanophages were isolated every month for 15 months on *Synechococcus* WH7803, using dilution-to-extinction coupled with plaque purification techniques. There was no evidence of multiple HGT events of *psb*A between cyanophages and their hosts. Phylogenetic analysis indicated that all of the *psb*A sequences obtained fell into the previously identified "Marine 1" cluster, containing marine myoviruses that infect *Synechococcus*. In contrast, there was evidence of recombination between cyanophage isolates. The majority (92%) of the 53 different OTUs identified clustered into eight well-supported clades. Recombination was determined by comparing the assigned clade based on the cyanophage isolates' *psb*A gene to that based on *g*20 (a conserved capsid assembly gene commonly used to characterize cyanophage phylogenetic diversity). This analysis indicated that ~ 1% of the isolates carried *psb*A sequences from a different clade than that of its *g*20 sequence. Finally, the abundance of the eight *psb*A clades varied substantially over time. In conclusion, *psb*A diversity in cyanophage coastal communities is temporally dynamic. Furthermore, homologous recombination of *psb*A among cyanophage clades appears to be a frequent occurrence, whereas cyanophage-host recombination does not.

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CT17.004 BROAD-HOST RANGE GENE TRANSFER PARTICLES PRODUCED BY *ALIIVIBRIO FISCHERI*

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Horizontal gene transfer (HGT) has played an important role in speciation and diversification of living organisms. We reported the novel "broad-host range vector particles: VPs", which are spontaneously released as budding particles without host cell death from some marine and/or thermophilic microbes. These VPs are distinctively different from the conventional viruses. We hypothesise that evolution of VPs is facilitate the host gene preservation along with the host cell growth.
This study was aimed to clarify characteristic the VPs of symbiotic luminous bacterium, *Aliivibrio fischeri* NCIMB1281T. It was grown in ZoBell broth 2216E at 20°C for 315 h with shaking at 60 rpm to produce spherical VPs from its logarithmic phase. After their growth period, the production of AfVP stabilised at around $7.0x10^{10}$-$7.4x10^{11}$ particles mL^{-1} without any change in host population counts. The basic characteristics of AfVPs are: diameter: 18.1-159.2 nm; buoyant density: 1.3607-1.3980 g cm^{-3}; and DNA size: 17.3-95.3 kb. Regardless of UV treatment, AfVPs enhanced the efficiency of plating to 116-136% at a multiplicity of infection (MOI) of *ca*. 140 in *Escherichia coli* AB1157. Non-UV treated AfVP gave high generalised transduction frequency of 10^{-4}-10^{-6} cells per particle. Upon infection, the particle-coat remained outside the cell, and a string-like structure entered the recipient. Generated *E. coli* transductant (AfV-E-trans) acquired the ability of budding particle production, and reached a growth maximum of *ca*. 415% of the parental *E. coli*. Unlike the release of VPs only after the hosts entered the stationary phase, the releasing of VPs in our bacterium was regardless of its growth phase.
We propose that the HGT by AfVP with a high MOI in nature aids to concomitant gene translocation and expression by non-virulent particles as an apparatus for host's gene preservation, whose trait is distinctive from the conventional virus concepts.

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CT17.005 GENEFISH: A WINDOW INTO TARGETED BACTERIAL DIVERSITY

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The interest for cultivation-independent molecular approaches that extract DNA directly from the environment is related to their capacity to overcome biases in isolation and in vitro cultivation of bacteria, thus providing new possibilities in the search for knowledge within the "black box" of soil microbiology. However, "classical" metagenomic methods are time- and money-intensive requiring construction and screening of million-clone DNA libraries to cover the huge bacterial diversity. Our objective is to develop a complementary method to the traditional metagenomic approaches, based on the use of a specifically engineered recipient *E.coli* strain to capture genes from soil bacteria after transformation with metagenome DNA. The so-called "Genefish" method is based on the use of specific sequences cloned into the recipient strain to serve as template for homeologous recombination with corresponding DNA present in the metagenome. In addition, the double cross-over event involving the targeted genes leads to the replacement of two inducible lethal genes that kill non-recombinant bacteria. Details of the *E.coli* construction including molecular tricks to precisely regulate expression of the lethal genes and of the lambda phage recombinase gene to increase recombination efficiency will be described as well as preliminary applications for the capture of the widespread nitrate reduction genes among soil bacteria. This "Genefish" technology, characterized by the positive screening of recombinant clones is used in the frame of the "Metaexplore" European project, involving 15 institutional and industrial expert groups from 11 countries as one of the technological and conceptual approach developed for discovering new enzymes with important industrial applications, such as chitinases, ligninases and dehalogenases.

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CT17.006 EVOLUTION AND NICHE SPECIALIZATION IN *VIBRIO* SPECIES. IS THE INTEGRON THE PERPETRATOR?

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Bacteria of the *Vibrio* genus are abundant in aquatic environments, fulfil important nutrient cycling roles and are often found in association with marine animals such as corals, molluscs, crustaceans and fish. The diverse roles that vibrios play emphasise their amazing ability to adapt and specialize into varied niches. Lateral gene transfer (LGT), the method by which DNA moves between bacterial cells is now known to be critical in the evolution of bacteria. The 'integron' is a genetic system present in the chromosome of ~10% of bacteria that aids in LGT by enabling the addition of mobile DNA into a dedicated genetic site. In all members of the *Vibrio* genus, the presence of integron-associated genes, contained within pieces of mobile DNA called 'gene cassettes' makes up a substantial component of the vibrio genome (1-3%). Poorly understood is the role that integrons play in vibrio evolution and niche specialization. This has been difficult to address since analysis of gene cassettes from sequenced vibrio genomes show that approximately 80% of gene cassette proteins have no significant similarity to existing protein sequences in GenBank. Thus, there is virtually no functional information for the vast majority of the integron/gene cassette proteins of *Vibrio* species. To address this knowledge gap, we have used homologus recombination to create large deletions in the integron cassette array of a marine isolate *Vibrio* sp. DAT722, which contains 116 gene cassettes. These deletion mutants combined encompass deletion of 65% of the total gene cassettes. One of these mutants is unable to alter outermembrane porins in response to shifts in osmolarity indicating an important role for a novel gene cassette product in the osmotic shock response. Proteomics are being conducted on these mutants to identify new phenotypes and determine whether gene cassette products interact with host proteins through regulation or post-translational modification.

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CT17.007 SHIFTS IN HOST RANGE OF A PROMISCUOUS PLASMID THROUGH PARALLEL EVOLUTION OF ITS REPLICATION INITIATION PROTEIN

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Plasmid-mediated gene transfer is an important means for rapid bacterial adaptation, as exemplified by the dramatic spread of multi-drug resistance. While the ability of plasmids to adapt to novel hosts and thereby shift their host range is key to their long-term persistence in bacterial communities, little is known about the evolutionary patterns of plasmid host range shifts. Our long-term goal is to understand if and how plasmids can expand, contract or shift their host range. The objective of this study was to determine the evolutionary dynamics of specialization of a promiscuous IncP-1β mini-replicon to a host wherein it was initially unstable. This was done by experimentally evolving *Shewanella oneidensis* MR-1 with plasmid pMS0506 for 1000 generations in serial batch cultures under antibiotic selection for plasmid maintenance. The plasmid stability was then examined at different time points and the mutations responsible for the change in phenotype were determined by resequencing evolved plasmids. Evolved plasmids showed improved stability in both the coevolved and ancestral host, indicating that adaptive mutations had occurred in the plasmid itself. Multiple unique plasmid genotypes were found that had various kinds of single genetic changes at one locus, which encodes the N-terminus of the replication initiation protein TrfA. All mutations tested resulted in increased host fitness under antibiotic selection, and most were responsible for a significantly higher plasmid copy number. Evolved plasmids had clearly shifted their host range, as they were no longer able to replicate in *Pseudomonas aeruginosa*. Moreover, genotyping of evolving populations suggested that clonal interference rather than single successive sweeps governed the evolutionary dynamics of plasmid-host adaptation. Our study shows that plasmid specialization to a single host can rapidly occur due to high plasticity in the *trfA* gene, and can result in a shift in plasmid host range.

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