Workshop on Plasmids as Vehicles of Antimicrobial Resistance Spread (smr 3761) Starts 21 Mar 2022 Ends 25 Mar 2022 - Online

Plasmids as vehicles of AMR spread

Tentative program

Overarching problem: Antimicrobial Resistance (AMR) represents a major challenge to public health worldwide. Mobile elements such as plasmids play a crucial role in Horizontal Gene Transfer (HGT) and AMR spread. Understanding plasmid evolution and mobility is fundamental to unravel the population genetics of plasmid-encoded genes, especially antibiotic genes.

Introductory talks

1. Bioinformatics background: going from raw data to plasmid genomes (Zamin Iqbal) Monday March 21st 3pm

2. Introduction to plasmid biology (Fernando de la Cruz) Wednesday March 23rd 3pm

3. Introduction to experimental evolution (Alejandro Couce Iglesias) Thursday March 24th 3pm

4. Plasmid Genetic epidemiology: why it is of paramount importance to step further than the standard species/resistance approach to track down plasmid encoded resistances. (Alice Ledda) Friday March 25th 3pm

- ***Plasmid-mediated horizontal gene transfer in microbiomes***

Mike Brockhurst, Katharine Coyte, Cagla Stevenson, Chris Knight, Jamie Hall, Ellie Harrison

Resistance genes are common in microbiomes but how their mobility affects the stability and dynamics of these communities is unknown. Using modelling and experiments we

show that plasmid-encoded resistance can increase the stability of the microbiome whilst the effect on the donor species is contingent upon the network of ecological interactions in the community. These findings suggest that plasmids (and other mobile elements) encoding resistance genes can have important effects on microbiome dynamics.

- ***Why do some bacterial genes reside on the chromosome and others on plasmids?***

Sonja Lehtinen

Bacterial genes can either reside on the chromosome or on plasmids, extrachromosomal genetic structures that can be transferred from cell to cell. The distribution of genes between plasmid and chromosome is not random: certain types of genes are particularly likely to be plasmid-associated. This includes a number of clinically important traits, such as antibiotic resistance and virulence factors. The evolutionary mechanisms that give rise to this pattern are not well understood. Plasmids are occasionally lost during cell replication and thus less reliably inherited than the chromosome, and genes are free to transition between plasmid and chromosome: so what keeps genes on plasmids? We address this question through mathematical modelling. The key insight from our model is that the relative fitness of chromosomal and plasmid-borne genes depends on their relative frequencies (positive frequency-dependent selection). In other words, the fitness of a plasmid-borne gene will be higher in a population in which the chromosomal gene is rare (and vice versa). This positive frequency dependence can keep moderately beneficial genes on plasmids, despite occasional plasmid loss. This leads to a priority effect: whichever form of the gene (i.e., plasmid-borne or chromosomal) is acquired first has time to increase in frequency and thus becomes difficult to displace. Therefore, the relative rate of acquiring the gene on the plasmid versus the chromosome predicts where the gene will be found. Further modelling shows this effect is particularly pronounced when genes are beneficial across a large number of species. All together, the hypothesis that emerges from our work is that plasmid-borne genes are moderately beneficial; functional across a large number of species; and rarely acquired through chromosomal mutation. We suggest traits like antibiotic resistance are often found on plasmids because these genes commonly fulfil these criteria.

- ***Characterising intracellular transposition events from plasmids to other replicons.***

Supathep Tansirichaiya1, Richard N. Goodman2, Adam P. Roberts2

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Antimicrobial resistance genes are distributed within and between bacterial populations via plasmids, on which they often reside within smaller mobile genetic elements (MGEs). In this study we aimed to develop a genetic system that would enable us to determine the intracellular transposition potential of different antimicrobial resistance genes present on these smaller MGEs.

We engineered an entrapment vector, called pBACpAK, to capture MGEs in Escherichia coli. pBACpAK contains a cI-tetA positive selection cartridge in which the cI gene encodes the CI repressor that inhibits the expression of tetA. Therefore, any disruption of cI, for example, by insertion of a MGE, will allow tetA to be expressed and result in a selectable tetracycline-resistance phenotype.

pBACpAK was introduced into both laboratory and clinical E. coli and into a recombination deficient, and MGE free, E. coli enabling us to directly link intracellular transposition to intracellular conjugation from other Enterobacteriaceae.

We have captured multiple insertion sequences, novel transposons, an entire multidrug resistance plasmid and have detected the de novo formation of potential composite transposons, integrated plasmids and off target transposition events giving us insights into the behaviour of independent transposable elements carried on plasmids following conjugation into a new host cell. This genetic system will allow us to carry out fine scale analysis of intracellular transposition events with a view to designing interventional strategies to interrupt these processes.

- ***The Darwinian fitness of extrachromosomal genetic element***

Tal Dagan

Plasmids are an important source of raw material for microbial genome evolution outside the mainstream of bacterial chromosomes. Nonetheless, many plasmids found in nature are lacking a clear trait that is advantageous to their host; the determinants of plasmid evolutionary success in the absence of plasmid benefit to the host remain understudied. Borrowing terminology from evolutionary biology of cellular living forms, we hypothesize that Darwinian fitness is key for the plasmid evolutionary success. In this talk I will present recent results from my group on the determinants of plasmid fitness, and how plasmid fitness might change depending on the environmental conditions.

- ***Chicken gut microbiome members limit the spread of an antimicrobial resistance plasmid in Escherichia coli***

Sarah Duxbury

Plasmid-mediated antimicrobial resistance is a major contributor to the spread of resistance genes within bacterial communities. Successful plasmid spread depends upon a balance between plasmid fitness effects on the host and rates of horizontal transmission. While these key parameters are readily quantified in vitro, the influence of interactions with other microbiome members is largely unknown. Here, we investigated the influence of three genera of lactic acid bacteria (LAB) derived from the chicken gastrointestinal microbiome on the spread of an epidemic narrow-range ESBL resistance plasmid, IncI1 carrying blaCTX-M-1, in mixed cultures of isogenic Escherichia coli strains. Secreted products of LAB decreased E. coli growth rates in a genus-specific manner but did not affect plasmid transfer rates. Importantly, we quantified plasmid transfer rates by controlling for density-dependent mating opportunities. Parametrization of a mathematical model with our in vitro estimates illustrated that small fitness costs of plasmid carriage may tip the balance towards plasmid loss under growth conditions in the gastrointestinal tract. This work shows that microbial interactions can influence plasmid success and provides an experimental-theoretical framework for further study of plasmid transfer in a microbiome context.

- ***Population size mediates the contribution of high-rate and large-benefit mutations to the evolution of drug resistance***

Joachim Krug

Abstract: The talk reports on evolution experiments showing that small and 100-fold larger bacterial populations evolve resistance to a β-lactam antibiotic by using similar numbers, but different types of mutations. Small populations frequently substitute similar high-rate structural variants and loss-of-function point mutations, including the deletion of a low-activity β-lactamase on a plasmid, and evolve modest resistance levels. Large populations more often use low-rate, large-benefit point mutations, including mutations activating the β-lactamase, leading to much higher resistance levels. The rates and effect sizes of different mutation classes are inferred from the endpoint genotypes using a machine learning approach, and an analytically solvable clonal interference model is presented that quantifies the shift from high-rate to large-benefit mutations with increasing population size.

- ***Role of plasmids and other mobile genetic elements in the broad-scale propagation of amino-glycoside resistances***

Authors: *Stéphanie Bedhomme, Léa Pradier*

Abstract: Aminoglycosides have been used to treat bacterial infections in humans since the 40's. Nowadays, they are only used in humans as a last-resort treatment for Gram-negative bacteria but they remain frequently used in agriculture and veterinary medicine. The main aminoglycoside resistance mechanism is through the production of aminoglycoside modifying enzymes (AME). In this study, over 160,000 Eubacteria genomes were screened for the presence of AME genes and for mobile genetic elements (plasmids, phage, transposons…) associated with them. This data set, combined with the genomes metadata, first permitted to get a picture of temporal, geographical and ecological propagation of these resistance genes. The use of an implicit phylogenetic method then allowed to reconstruct horizontal gene transfer (HGT) networks and to decipher the horizontal traffic rules for these genes. These analyses highlighted the role of mobile genetic elements, and in particular of plasmids, (1) in the accumulation of resistance genes within genomes and the broadening of the resistance spectrum and (2) as vehicles bypassing barriers to HGT such as phylogenetic distance or codon usage differences between donor and receiver genomes.

- ***Plasmid invasion in a mathematical multispecies model of conjugation***

Jesse Alderliesten

Department of Population Health Sciences, Utrecht University, the Netherlands

Most mathematical models that are used to study the spread of plasmid-based antibiotic resistance consider only a single bacterial species, and models that consider multiple species usually incorporate only a single conjugation rate to describe conjugation within and between the different species. Models that incorporated multiple conjugation rates have been used to explain how plasmids that are unable to persist in a monoculture of an inefficient donor are able to persist in the donor if the donor is cocultured with plasmid-free strains that are efficient donors. These models did not incorporate interspecies interactions. However, interspecies interactions can change the abundances of the various species, thereby facilitating or hampering plasmid spread between species. In addition, interspecies interactions in a healthy microbiome prevent colonization by exogenous microorganisms. To take these effects into account, we created a multispecies model of conjugation that incorporated interspecies interactions and multiple conjugation rates. We used this model to investigate how the possibilities for a plasmid to invade and spread through the microbiome are influenced by the presence or absence

of differences in conjugation rates, by interspecies interactions, by the introduction of the plasmid through a species that either was already present or was not yet present in the microbiome and by the number of species that is present in the microbiome.

- ***Estimating plasmid conjugation rates***

Jana Huisman

Abstract: "Plasmids are important vectors for the spread of genes among diverse populations of bacteria. However, there is no standard method to determine the rate at which they spread horizontally via conjugation. Here, we compare commonly used methods on simulated and experimental data, and show that the resulting conjugation rate estimates often depend strongly on the time of measurement, the initial population densities, or the initial ratio of donor to recipient populations. Differences in growth rate, e.g. induced by sublethal antibiotic concentrations or temperature, can also significantly bias conjugation rate estimates. We derive a new 'end-point' measure to estimate conjugation rates, which extends the well-known Simonsen method to include the effects of differences in population growth and conjugation rates from donors and transconjugants. We further derive analytical expressions for the parameter range in which these approximations remain valid. We present an easy to use R package and web interface which implement both new and previously existing methods to estimate conjugation rates. The result is a set of tools and guidelines for accurate and comparable measurement of plasmid conjugation rates."

- ***Understanding the impact of host contact dynamics on pathogen diversity***
  Chiara Poletto

Abstract:
The co-circulation among multiple pathogen strains affects the spread of infectious diseases and the efficacy of interventions. Genomic tools have made it increasingly easy to observe pathogenic strains diversity, but the best interpretation of such diversity has remained difficult because of relationships with host and environmental factors. During my talk I will focus on host-to-host contact behavior and use stochastic modeling and network theory to quantify its effect on strains' co-circulation. I will discuss how contact heterogeneities reduce the diversity of competing strains by limiting the number of circulating strains and leading a few strains to dominate over the others. When the interactions among strains are heterogeneous, the interplay between strain traits and the properties of the host contact network may lead to a complex co-existence/dominance

diagram. These results provide fundamental understandings that may help interpreting observations.

- ***A meta population model preserves the genomic diversity in presence of gene sweeps***

Marco Cosentino Lagomarsino

The horizontal spreading of genes is at the basis of the the eco-evolutionary processes taking place in microbial communities of ecological importance, such as oceanic plankton, soil, and human microbiome. Several hypotheses were put forward to explain the experimentally documented process of "gene sweeping", whereby a beneficial gene spreads in a bacterial community maintaining biodiversity. Shapiro and colleagues, who first found direct experimental evidence for this process, have proposed a mechanism of decrease of the flow of genes between increasingly ecologically distinct populations. Kaneko and collaborators have proposed instead that ubiquitous deleterious loci linked with the beneficial one may lead to gene sweeps by means of frequency-dependent selection. Finally, Niehus and coworkers showed the possibility of maintaining biodiversity in a single population in the presence of very high rates of horizontal gene transfer. Here, we propose a different mechanism, based on the meta-population structure of a community. We show that a meta-population structure maintains biodiversity upon arrival of a sweeping beneficial gene. Our model predicts the main time scales involved in gene sweeping in meta populations, based on key parameters such as migration and horizontal transfer rates.

- ***Diversity and transmission of ESBL plasmids in Klebsiella pneumoniae in the hospital setting***

*Jane Hawkey*1, Kelly L Wyres*1, Louise M Judd1, Ryan R Wick1, Claire L Gorrie2, Adam W J Jenney3, Kathryn E Holt1,4*

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Healthcare-associated infections are a top global health priority. Genomics is a powerful approach for investigating transmission of antimicrobial resistant (AMR) infections, however most genomics studies focus on strain, rather than plasmid, transmission. From a collection of 104 third-generation cephalosporin resistant K. pneumoniae isolates collected across one year in a large Australian hospital, we used long-read sequencing to complete 70 extended-spectrum beta-lactamase (ESBL) plasmids. We developed methods to detect plasmid transmission events, and found 25 distinct ESBL plasmids in our collection. The majority of ESBL burden during the study period was due to a single plasmid associated with a clonal expansion of K. pneumoniae ST323, plus plasmid transmissions into four additional K. pneumoniae strains, two of which subsequently underwent clonal expansion to persist in the hospital for at least four years. These methods that allow for surveillance of plasmid transmission are key for aiding prevention of transmission of AMR pathogens.

- ***Plasmid overlap and evolution between Enterobacterales isolates from bloodstream infections and non-human compartments***

William Matlock1, Samuel Lipworth1,2, A Sarah Walker1,4,5, Derrick Crook1,2,4,5, Daniel Read6, Muna Anjum7, Liam P Shaw3/Nicole Stoesser1,2,4,5, REHAB Consortium

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Background and aims: The extent to which important species of Enterobacterales and mobile genetic elements are shared across human and non-human compartments remains poorly understood. While some previous studies have found limited evidence of genetic overlap amongst Enterobacterales causing human infections and those from non-human reservoirs, these have often been: (i) limited in size given the genetic diversity in these niches, (ii) restricted to single species or phenotypes (e.g. drug-resistant isolates), and (iii) have not fully evaluated the dissemination of mobile genetic elements, such as plasmids. To explore Enterobacterales plasmid diversity and sharing across reservoirs in a geographically restricted context, we studied large Enterobacterales isolate collections from human bloodstream infections (BSI; Oxfordshire, UK, 2008-2018), and from

longitudinal sampling of livestock (cattle, pigs, poultry, sheep), wastewater (influent, effluent) and rivers (Oxfordshire, 2017).

Methods: All cultured isolates were sequenced on Illumina and Oxford Nanopore Technologies' sequencers; hybrid assembly (Unicycler) was used to recover genomes (chromosomes and plasmids). Plasmids were clustered by k-mer Jaccard similarities (Mash), then cluster pangenomes were constructed (Panaroo). We built phylogenies (IQ-Tree) based on core gene alignments to investigate the phylogenetic structure of plasmid clusters alongside their accessory gene content, then time-scaled these phylogenies (LSD2 in IQ-Tree).

Results: The combined dataset contains n=1,458 complete Enterobacterales genomes, including n=3,697 circularised plasmids. 2,627/3,697 plasmids segregated into n=247 genetically related clusters of at least 3 plasmids. Of these, n=69 clusters contained over 10 members (1,832/3,697 plasmids), which were used for subsequent pangenomestyle analyses. Clusters showed high concordance with replicon haplotype (purity=0.89), host genera (purity=0.95), and predicted mobility (purity=0.97). 73/247 (30%) clusters contained plasmids from both human BSI and nonhuman niches. Pangenome analyses of plasmid clusters showed different accessory gene repertoires carried within conserved plasmid 'backbones'. In 5/69 clusters investigated so far, we have seen n=6/8 sub-clusters containing plasmids from human BSIs and non-human reservoirs, sharing highly similar plasmid backbones (no. nucleotide substitutions per site <0.00001) and concordant accessory gene repertoires. Preliminary phylogenetic dating of these sub-clusters suggests t-MRCAs between years and decades.

Conclusions: Our data highlights that plasmid sharing across human and non-reservoirs in Enterobacterales is not uncommon but may require dense sampling to detect. Different plasmid clusters have different dynamics, including the extent to which they are shared across reservoirs, species of Enterobacterales and species sub-lineages. Our analysis supports across-niche movement of plasmids relevant to human infection in recent evolutionary history, including those carrying important antimicrobial resistance genes.

- ***Rapid spread and evolution of plasmid-mediated antibiotic resistance in the gut microbiota of hospitalized patients***

Alvaro San Millan

Antimicrobial resistance (AMR) in bacteria is one of the most serious threats to public health worldwide. The gut microbiota of hospitalized patients is one of the most important hotspots of AMR dissemination and evolution in hospitals, and conjugative plasmids–DNA molecules able to transfer horizontally between bacteria – play a key role in this process. The factors that drive the evolution of plasmid-mediated AMR have been studied

in detail over the last years, using different in vitro experimental approaches with a wide variety of plasmid-bacteria associations. However, the in vivo evolutionary dynamics of plasmid-mediated AMR remain largely unexplored. Over the last years, we have studied the spread and evolution of plasmid-mediated AMR in the gut microbiota of hospitalized patients. We focused on the widespread carbapenemase-encoding plasmid pOXA-48, which is one of the most relevant plasmids in clinical settings across Europe. We used a collection of 250 pOXA-48-carrying enterobacteria isolated from 150 hospitalized patients in a large tertiary hospital in Madrid over a period of two years. First, we analyzed the between- and within-patient plasmid transmission dynamics, revealing the pervasive conjugation of pOXA-48 in the gut microbiota of patients. Next, we investigated pOXA-48 evolution in the hospital, analyzing the phenotypic effects of the different mutations observed in the plasmid across the 250 isolates. Finally, we identified and characterized three different examples of intra-patient evolution of pOXA-48-mediated resistance. Our study revealed that a trade-off between resistance and fitness shapes the rapid evolution of plasmid-mediated carbapenem resistance in the gut microbiota of hospitalized patients.

- ***Experimental evolution of highly conjugative plasmids and their effects on AMR spread***

Tatiana Dimitriu

AMR plasmids spread through populations due to a combination of vertical and horizontal transmission. When evolved in the presence of abundant susceptible hosts, a condition which favours horizontal transmission, the AMR plasmid R1 rapidly evolves mutations causing increased plasmid copy number. High copy number in turn increases both horizontal transmission rates and the level of AMR conferred by plasmid genes. However, plasmid variants with deletion of the full antibiotic resistance region also evolve. High copy number is more frequent in these deleted plasmid variants, allowing them to outcompete the ancestral AMR plasmid and lead to the loss of AMR observed at the population level. Deleted plasmids efficiently counter both vertical and horizontal transmission of the ancestral AMR plasmid, suggesting they could be used to limit the spread of AMR.

- ***Using pangraph to explore plasmid diversity***

Liam Shaw

Pangraph (Noll et al., biorxiv 2022) is a recent tool for rapidly aligning genomes into a graph data structure. While it was developed with the aim of application to whole genomes, it is ideally suited for exploring the structural diversity of plasmids. I will talk about some work-in-progress experimenting with pangraph on plasmid datasets.

- ***New Approaches to Understanding Gene Content in prokaryotic Pangenomes***

James McInerney

Prokaryotes live their lives under intense pressure from phage and protozoan predation, they can experience frequent ecological upheaval, and encounter competition for food and energy from both closely and distantly related organisms. Horizontal gene transfer and gene loss in these kinds of dynamic environments has resulted in pangenomes forming in prokaryotic (and eukaryotic) genomes. Despite the pangenome phenomenon being discovered almost two decades ago, and with >1,000 publications on pangenomes and >10,000 publications mentioning pangenomes, it remains one of the few areas of evolutionary biology where empirical observations of the phenomenon are not accompanied by a comprehensive body of theory for how pangenomes arise and are maintained. I argue here that external existential threats, as well as opportunities have created the conditions for the evolution of dynamic prokaryotic pangenomes that have their own ecological rules. As an analogy, Prokaryotic pangenomes can be said to approximate situations that are normally associated with macroecology, however, in this case the interacting and non-interacting ecological actors are genes within pangenomes, and not whole organisms living on a savannah, or in a swamp. I will argue that pangenomes manifest evidence of extensive competition, co-operation, interdependence and antipathy. We are a long way from having a robust theoretical framework, supported by empirical evidence, for understanding pangenomes, but progress is possible if we view pangenomes through the proper lens.

- ***High-resolution sequence annotation and comparative analyses as foundations for plasmid surveillance***

Robert A. Moran

It is important to define and characterise individual plasmid lineages in order to study their ongoing evolution and facilitate sensitive genomic surveillance. Plasmids, particularly those associated with antibiotic resistance, are shaped by the actions of translocatable elements such as insertion sequences and transposons. When translocatable elements insert in new positions or mediate deletions they generate unique element-target junction sequences characteristic of those specific molecular events. Combinations of junction and other typing sequences can be used to identify representatives of plasmid sub-lineages that have been defined by their recent evolutionary histories through comparative analyses. This enables studies of the dissemination and diversification of discrete plasmid lineages.

In the DETECTIVE project we have been studying ESBL and carbapenem-resistant Gram-negative pathogens from Chinese intensive care units (ICUs) since 2019. This talk will describe the approaches used to examine plasmids from our Escherichia coli, Klebsiella pneumoniae and Acinetobacter baumannii collections, and to study their distribution and evolution at local and international scales. These approaches have resulted in the characterisation of internationally distributed F-, I- and L/M-complex plasmid lineages carrying antibiotic resistance genes in members of the Enterobacterales, and the detection of cryptic Aci6-type plasmid transfer events in a single ICU's A. baumannii population. The talk will particularly focus on methods for the accurate annotation and comparison of plasmid backbone and translocatable element sequences, identification of informative sequence junctions, and the application of junction sequences to large whole-genome datasets. Complex Acinetobacter plasmids that appear to evolve through XerC/D-mediated recombination events and require unusual annotation approaches will be discussed briefly.

- ***Estimating the rate of plasmid transfer with an adapted Luria-Delbrück fluctuation analysis***

Olivia Kosterlitz

To increase our basic understanding of the ecology and evolution of conjugative plasmids, we need a reliable estimate of their rate of transfer between bacterial cells. However, accurate estimates of plasmid transfer have remained elusive due to biological and experimental complexity. Current methods to measure transfer rate can be confounded by many factors. A notable example involves plasmid transfer between different strains or species where the rate that one type of cell donates the plasmid is not equal to the rate at which the other cell type donates. Asymmetry in these rates has the potential to bias or constrain current transfer estimates, thereby limiting our capabilities for estimating transfer in microbial communities. Inspired by the classic fluctuation analysis of Luria and Delbrück, we develop a novel approach, the Luria-Delbrück method ('LDM'), for estimating plasmid transfer rate. Our new approach embraces the stochasticity of conjugation departing from the current deterministic population dynamic methods. In addition, the LDM overcomes obstacles of traditional methods by not being affected by different growth and transfer rates for each population within the assay. Using stochastic simulations and experiments, we show that the LDM has high accuracy and precision for estimation of transfer rates compared to the most widely used methods, which can produce estimates that differ from the LDM estimate by orders of magnitude.

- ***PanGraph: scalable bacterial pan-genome graph construction***

Marco Molari

The genomic diversity of microbes is commonly parameterized as population genetic polymorphisms relative to a reference genome. However reference genomes often contain only a fraction of the microbial pangenome, the set of genes observed within all isolates of a given species, and are thus blind to both the dynamics of the accessory genome, as well as variation within gene order and copy number.

With the wide-spread usage of long-read sequencing, the number of high-quality, complete genome assemblies has increased dramatically. Traditional computational approaches towards whole-genome analysis either scale poorly, or treat genomes as dissociated bags of genes, and thus are not suited for this new era.

In this short talk I will present PanGraph, a Julia based library and command line interface for aligning whole genomes into a graph, wherein each genome is represented as an undirected path along vertices, which in turn, encapsulate homologous multiple sequence alignments. The resultant data structure succinctly summarizes population-level nucleotide and structural polymorphisms and can be exported into a several common formats for either downstream analysis or immediate visualization.

- ***Plasmids in plant and soil associated bacteria***

Kornelia Smalla

Julius Kühn-Institut Federal Research Centre for culivated plants, Braunschweig

Antibiotic resistance threatens the achievements of modern medicine and insights on the dissemination routes and the factors that foster the evolution of the transferable resistome are urgently needed. Plasmids are assumed to play a major role in the dissemination of antibiotic resistance genes (ARGs) but very few studies focused on plasmids. The potentials and limitations of a range of cultivation-dependent and independent methods to detect ARGs and mobile genetic elements (plasmids, integrons) will be discussed. Data on the abundance of ARGs and plasmids in total community DNA from various field-scale organic fertilizers by PCR-Southern blot hybridization, qPCR and high throughput-qPCR will be presented. The presence and transferability of plasmids was confirmed by exogenous capturing of plasmids conferring antibiotic resistances. We showed that sewage sludge from ten different sewage treatment plants contained besides nutrients antibiotic, heavy metals and disinfectant residues and bacteria carrying plasmid localized resistance genes. Spread organic fertilizer had strong but transient effects on the

abundance of ARGs and plasmids as well as on the soil prokaryotic communitiy composition that were more pronounced in bulk soil compared to the rhizosphere.

Culture dependent and -independent approaches were employed to assess the transferable resistome of bacteria associated with produce and a remarkable diversity of self-transmissible antibiotic resistance plasmids assigned to the IncF, IncI, IncN and IncP, was detected in rare microbiome (E. coli isolates) associated with raw-consumed produce. While the quantification of the transferable resistome in total community-DNA failed due to the low abundance in the produce microbiota, the transferable resistome became detectable in DNA extracted after nonselective enrichment.We show that the transferable resistome is often occurring only in the rare microbiome. Sensitivity of detection is an important issue in particular for cultivation independent methods. Thus in order to understand the ecology and dissemination of plasmid-mediated antibiotic resistances, a polyphasic approach also including enrichments is recommended.

- ***Plasmid Taxonomy***

Fernando de la Cruz.

Institution:

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Abstract:

Plasmids mediate horizontal gene transfer of antibiotic resistance, virulence genes, and other adaptive factors across bacterial populations. We analyzed the genomic composition and pairwise sequence identity for about 20,000 reference plasmids from RefSeq200, to obtain an updated global map of the prokaryotic plasmidome, thus expanding our previous map (1). Now we analyze the structure and dynamics of the main plasmid taxonomic units (PTUs), analogous to bacterial species. Furthermore, we developed an automated algorithm for PTU assignation, called COPLA (2). We would like to propose a hierarchical taxonomic classification of plasmids that includes classes (homologous MOB), families (homologous TRA) and PTUs (homologous backbone). The interest of this classification will be discussed.

References:

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