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1

"Population genomics of aquatic prokaryotes"

Francisco Rodriguez-Valera
E-mail: frvalera@umh.es. Univ. Miguel Hernández. Departamento de Producción Vegetal y Microbiología. Carretera de Valencia s/n km 8.6.

We have examined a collection of the free-living marine bacterium*Alteromonas* genomes with cores diverging in average nucleotide identities (ANIs) ranging from 99.98 to 73.35%, i.e. from microbes that can be considered members of a natural clone (like in a clinical epidemiological outbreak) to borderline genus level. The genomes were largely syntenic allowing a precise delimitation of the core and flexible regions in each. The core was 1.4Mb (*ca*. 30% of the typical strain genome size). Recombination rates along the core were high among strains belonging to the same species (37.7 to 83.7% of all nucleotide polymorphisms) but they decreased sharply between species (18.9 to 5.1%). Regarding the flexible genome, its main expansion occurred within the boundaries of the species, i.e. strains of the same species already have a large and diverse flexible genome. Flexible regions occupy mostly fixed genomic locations. Four large genomic islands are involved in the synthesis of strain-specific glycosydic receptors that we have called glycotypes. These genomic regions are exchanged by homologous recombination within and between species and there is evidence for their import from distant taxonomic units (other genera within the family). In addition, several hotspots for integration of gene cassettes by illegitimate recombination are distributed throughout the genome. They code for features that give each clone specific properties to interact with their ecological niche and must flow fast throughout the whole genus since they are found, with nearly identical sequences, in different species. Models for the generation of this genomic diversity involving phage predation are discussed.

2

"Metagenomic Recovery of Phage Genomes of Uncultured Freshwater Actinobacteria"

Rohit Ghai[1*], Maliheh Mehrshad[2,3], Carolina Megumi Mizuno[2,4], Francisco Rodriguez-Valera[2]

Email: ghai.rohit@gmail.com. *[1]Institute of Hydrobiology, Department of Aquatic Microbial Ecology, Biology Center of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic. [2]Evolutionary Genomics Group, Universidad Miguel Hernandez, Alicante, Spain. [3]Extremophiles Laboratory, Department of Microbiology, Faculty of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran. [4]Department of Microbiology, Unité Biologie Moléculaire du Gène chez les Extrêmophiles, Institut Pasteur, Paris 75015, France*

Low-GC Actinobacteria are among the most abundant and widespread microbes in freshwaters. Some of them are also among the smallest, most streamlined bacteria in these habitats. Despite their ubiquitous presence in freshwaters all over the world, they have largely resisted cultivation efforts. Consequently, the nature of their viral predators, that are likely to serve as primary agents in preserving the diversity of these populations, have remained totally unknown. In this work, we have used deep metagenomic sequencing to assemble eight complete genomes of the first tailed phages that infect freshwater Actinobacteria. Their genomes encode the actinobacterial-specific transcription factor whiB, frequently found in mycobacteriophages and also in phages infecting marine pelagic Actinobacteria. This finding suggests a common and widespread strategy of modulation of host transcriptional machinery upon infection via this transcriptional switch. We also present evidence that these whiB-carrying phages infect the acI lineage of Actinobacteria. Moreover, at least one of them encodes the ADP-ribosylating component of the widespread family of AB-toxins (e.g. clostridial toxin), and we posit that the presence of this toxin reflects a „trojan horse" strategy, providing a generic protection to the abundant host microbes at the population level against their eukaryotic predators.

3

"Haemodyalisis water microbiome"

Margarita Gomila, Claudia Prince, Antonio Busquets, Elena García-Valdés, Jorge Lalucat
E-mail: marga.gomila@uib.es. Microbiología – Departamento de Biología; Universitat de les Illes Balears, Ctra. Valldemossa, Km 7.5; 07122 Palma de Mallorca (Spain) and IMEDEA (CSIC-UIB)

The microbiological quality of water is of crucial importance in the renal replacement therapy of patients undergoing haemodialysis. The water used to prepare haemodialysis fluid constitutes an oligotrophic habitat in which complex bacterial communities with a high level of diversity can develop. The microorganisms present in water can form biofilms on filters, tanks and water pipes which hinders their elimination and facilitates their persistence. Some of these bacteria are potentially pathogenic, may be the source of nosocomial infections, and act as a reservoir and medium for the dissemination of antibiotic resistance genes. In addition, some microbial products resulting from the lyses and death of microorganisms, as well as "nanobacteria", can pass through the haemodialysis filters, causing inflammatory reactions in patients with chronic renal failure. The presence of bacteria and bacterial-derived products (*i.e.,* endotoxins, sphingolipids, and oligonucleotides) can contribute to silent chronic inflammation, secondary amyloidosis, pyrogenic reactions and anemia.

The aim of this work is to expand our knowledge of the bacteria responsible of the biofilm in haemodialysis waters (Gomila *et al.*, 2005, 2006), by using next-generation sequencing methodologies and new strategies for identifying bacterial isolates. The main objective is to characterize the microbial community present in the haemodialysis water used to reconstitute the dialysis fluid at the hospital. The ultimate goal is the knowledge of the bacteria involved and the conditions that favour its development which will allow the establishment of methodologies for their monitoring and removal, getting a better haemodialysis water quality and contributing to the quality of life of persons subjected to this treatment.
The results obtained show that bacterial community present in the dialysis water is very complex, with microorganisms highly diverse and adapted to the oligotrophic habitat, not usually detected routinely in clinical microbiology laboratories.

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4

"The drugs within: Search for new probiotics and bioactive compounds in humans"

Alex Mira Obrador.
E-mail: mira_ale@gva.es. FISABIO. Fundación para el Fomento de la Investigación Sanitaria y Biomédica. Laboratorio de Microbioma Oral. Avda. de Catalunya, 21 / 46020 Valencia, España

Our body can be the source for new compounds and beneficial bacteria of biomedical interest. An example can be provided by our work on tooth decay. By performing metagenomic studies by direct DNA sequencing of the oral microbiota in caries-free and caries-active individuals we were able to identify a new bacterial species associated to individuals with good oral health, which we have named *Streptococcus dentisani*. This bacterium produces bacteriocins that inhibit the growth of several oral pathogens and is also able to buffer extracellular pH. Given that tooth decay is caused by acid production as a consequence of sugar fermentation by oral pathogens, *S. dentisani* is a promising probiotic to promote oral health. In a second metagenomic approach in which dental plaque DNA from caries-free individuals was cloned and expressed in *E. coli*, 10 DNA segments were found to inhibit the growth of the oral pathogen *Streptococcus mutans*. Four of them corresponded to bacterial compounds and six of them to DNA regions of the human genome, suggesting the identification of antimicrobial peptides of human origin. A similar strategy was performed in individuals that never developed flu symptoms despite high probability of being exposed to the virus (health care professionals), which led us to identify several human DNA segments with capacity to inhibit hemoagglutination of the H1N1 flu virus with red blood cells. Thus, we propose that healthy individuals with apparent immunity to infectious diseases can be the source of new anti-microbial compounds.

5

"Horizontal transfer of virulence factors present in Genetic Mobile Elements of *Staphylococcus aureus* in patients with Cystic Fibrosis"

Katerina Guzman[1], Mercedes Cervera[1], Miglè Ziemytè[1], Miguel Martí[4], Alicia Hernández[3], Amparo Solé[1,2] y Mª Ángeles Tormo-Mas[1].

E-mail: tormo_man@iislafe.es. [1] Grupo de Infección Grave. Instituto de Investigación Sanitaria la Fe. Valencia. [2]Unidad de Fibrosis Quística adultos. Servicio de Microbiología. Instituto de Investigación Sanitaria La Fe. [3]Departamento de Microbiología. Hospital Universitario y Politécnico la FE. [4] Facultad de Veterinaria y Ciencias Experimentales. Universidad Católica de Valencia "San Vicente Mártir"

S. aureus is an important pathogenic bacteria associated with a huge variety of infections and multidrug-resistance. The enormous versatility of *S. aureus* as a pathogen is due to its ability to persist and multiply in different environments along with its ability to produce a wide variety of virulence factors, most of which are encoded by mobile genetic elements (MGE), such as bacteriophages and pathogenicity Islands (Malachowa y DeLeo, 2010). One of the important virulence characteristics of *S. aureus* is its ability to form biofilm. Biofilms not only play an important role in the pathology of some chronic infectious diseases, but they also constitute an important environmental reservoir of pathogenic microorganisms (Lister JL *et al*., 2014). The formation of biofilms by Staphylococci has been associated with various chronic infections like cystic fibrosis (CF).

CF is a multisystem disease, but respiratory manifestations are the leading cause of morbidity and mortality, so that combating infectious exacerbations is one of the main strategies for the treatment of these patients. *S. aureus* is one of the most common pathogens of CF patients particularly in patients of young age (Goss CH *et al*., 2011). The ability of biofilm formation is critical in this type of infections favouring that patients are colonized persistently and therefore often under antibiotic therapy. Many antibiotics induce the SOS response, which triggers the induction of bacteriophage residents and as a result the induction of SaPIs. Therefore, this leads to an increase in the horizontal transfer of MGE and their associated virulence factors. Focusing on CF, the respiratory tract represents a mixing ground for multiple microorganisms, favouring a greater bacterial exchange, which enables the acquisition of MGE, which would enable it to adapt and persist in this specific niche.

For this reason, we have isolated 116 strains from different FQ patients during two years, classified by MLST typing and analysed their ability to form biofilm, by other hand we studied the presence of MGE (bacteriophages and pathogenicity Islands), and characterised known virulence factors encoded by this elements. Finally we selected and sequenced 22 strains to discovered new virulence factors present in these strains in order to increase our knowledge on how *S. aureus* adapts to this specific niche. It is feasible that these new factors may represent novel therapeutic targets. In addition, we want to identify new genes related to the formation of biofilm that may be useful as targets for inhibition of biofilm formation, or to identify enzyme activities capable of degrading biofilm matrix.

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6

"Studies on paralogous genes in *Myxococcus xanthus*"

Juana Pérez Torres, Muñoz Dorado
E-mail: jptorres@ugr.es; jdorado@ugr.es. Departamento de Microbiología. Universidad de Granada. Facultad de Ciencias. Avenida de la Fuente Nueva s/n. C.P. 18071, Granada, Spain

Myxococcus xanthus exhibits a life cycle with several multicellular behaviors, such as strategies for group predation or development. Myxobacteria hold the largest bacterial genomes published to date. Several studies seem to indicate that bacteria living in complex and changing environments have more internal and external opportunities to expand their genomes, and all the data point to the direction that a large genome is the result of developmental complexity and adaptation to variable environments. This is in agreement with the fact that the expansion of myxobacteria genomes have arisen largely through gene duplications of specific signaling genes families, particularly those involved in cell-cell signaling and signal transduction, which are likely to function in cell-cell interactions to maintain multicellularity and in response to their changing environment conditions, including the presence of prey for cooperative predation or starvation for development. We are studying the expansion of genes belonging to two different regulatory protein groups: i) the serine/threonine protein kinases (STPKs) and ii) the regulators implicated environmental responses, such as the presence of copper and other metals. STPKs are expected to be implicated in *M. xanthus* development because processes such as cell cycle and sporulation need direct temporal progression through multiple stages. We have discovered that four duplicated pairs of STPKs are implicated in formation of fruiting bodies and in phase variation. Transcriptomic analysis of the wild type strain and a mutant with deletion in the eight genes encoding STPKs during a complete developmental process will clarify the regulatory pathways controlled by these kinases. On the other hand, metal-stress responses need fast signal propagation, with short regulatory cascades. We have described the mechanism of actions and metal specificity of two paralogous genes coding for a novel group of metal-dependent extracytoplasmic sigma factors (ECFs) and several two component systems. Finally, we are also studying other aspects of *M. xanthus* multicellular behavior, such as cooperative predation and the consequences of the predator-prey interaction.

7

"Genome-wide identification of *Vibrio vulnificus* genes critical for pathogen replication in serum"

Carda-Diéguez M[1,2], Hor, L-I[3], Hubbard T[4,5,6], Chao MC[4,5,6], Waldor, MK[4,5,6] and Amaro, C[1,2].

E-mail: miguel.carda@uv.es. [1] Department of Microbiology and Ecology, University of Valencia. Dr. Moliner 50, 46100 Burjassot, Spain. [2] Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina (ERI BIOTECMED), Universitat de València. Dr Moliner 50, 46100 Burjassot, Spain. [3] Institute of Basic Medical Sciences and Department of Microbiology and Immunology, National Cheng-Kung University, Tainan, Taiwan, Republic of China. [4] Division of Infectious Disease, Brigham and Women's Hospital, Boston, Massachusetts, United States of America. [5] Howard Hughes Medical Institute, Boston, Massachusetts, United States of America. [6] Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, United States of America

V. vulnificus is an aquatic opportunistic pathogen commonly isolated from temperate, subtropical and tropical climates from both estuarine waters and marine animals (fish, oysters, sediment, shrimp and clams). This species can infect humans either through exposure of wounds to seawater or carrier-animals, or by ingestion of raw seafood The number of human infections caused by *Vibrio vulnificus* has increased in the last years, probably as a consequence of global warming that is extending its distribution to Northern countries. The rapidity with which the pathogen spreads from the infection site (skin or epithelial intestine) to the blood and the high morality (50%-90%) associated with septicemia are two of the properties that make this organism particularly frightening. We hypothesized that the capacity of the pathogen to proliferate in human serum would be critical to cause severe human disease. In this study, we carried out a genome-wide transposon insertion sequencing (TIS) screen to identify the genes that enable *V. vulnificus* YJ016 (a clinical isolate representative of the most virulent phylogenetic lineage) to survive and multiply in human serum. YJ016 survived and even efficiently multiplied in human serum. As predicted, our TIS screen yielded 8 genes belonging to a previously described capsule biosynthetic locus. The identification of capsule biosynthesis genes validates our approach, since the capsule is known to be critical for *V. vulnificus* serum resistance. Moreover, we identified 4 genes important for *V. vulnificus* proliferation in serum that are not known to be involved in capsule biosynthesis. We are currently investigating the role of these genes in serum resistance and virulence.

8

"Genomic analysis of outbreaks: the amazing *Legionella* from Alcoi (Spain)"

Fernando González-Candelas, Leonor Sánchez-Busó, Iñaki Comas
E-mail: fernando.gonzalez@uv.es. Unidad Mixta "Infección y Salud". FISABIO-Universidad de Valencia. CIBERESP. Spain.

Legionella pneumophila is a strictly environmental pathogen and the etiological agent of legionellosis. It is known that non-vertical processes play a major role in the long- and short-term evolution of pathogens but little is known about the relevance of these and other processes in environmental bacteria. We have used next generation sequencing to obtain nearly complete genome sequences of 69 *L. pneumophila* strains linked to recurrent outbreaks in a single location (Alcoy, Spain) along 11 years.

Many (n=45) of these isolates belong to the ST578, usually found in clinical samples from this location but rarely isolated from environmental samples or from other locations. Despite the relatively short time and the limited geographical area of sampling we found that none of these isolates was identical to any other. The number of SNPs along the complete genomes spanned between a minimum of 5 to a maximum of 1807 and were not related to the dates or outbreak when they had been isolated. In fact, several cases of larger intra-outbreak and inter-outbreak differences were observed among these isolates, thus rejecting the assumed clonal nature of *Legionella* outbreaks.

When we applied our method to detect recombination, we were able to identify 16 recombination events from other unidentified *Legionella* STs. These events had introduced most of the genetic variation, about 98% of the 2202 SNPs found in these isolates. After their removal, the estimated time for the colonization of the Alcoy area by this ST was 1993 with the 16 recombination events having occurred after that time. The estimated rate of evolution for this *L. pneumophila* ST was 1.39x10E-7 s/s/y (95% HPD 5.41x10E-8 to 2.30x10E-7 s/s/y), substantially lower than the estimate obtained without removing recombination (8.02x10E-6).

In consequence, recombination plays a major role in the short-term evolution of *L. pneumophila* with significant impact even in contemporaneous populations. These results have profound implications for our understanding of microbial populations and for public health interventions in *Legionella* outbreak investigations.

9 (S)

"*Mycobacterium llatzerense* genome"

Margarita Gomila, Daniel Jaén-Luchoro, Jorge Lalucat, Antoni Bennasar-Figueras
E-mail: jlalucat@uib.es. Microbiología – Departamento de Biología; Campus UIB - Ctra. Valldemossa, Km 7.5; 07122 Palma de Mallorca (Spain) and IMEDEA (CSIC-UIB)

Mycobacterium llatzerense is a rapidly growing *Mycobacterium* (RGM), also known as non-tuberculous mycobacteria (NTM). Species in this group are widely distributed in the environment and some of them are considered as emerging opportunistic pathogens. Nosocomial infections caused by RGM are usually difficult to treat as a consequence of the resistance to antibiotics or other external factors. *M. llatzerense* strain MG13^T was first isolated from pure haemodialysis waters and is characterised by its ability to grow facultatively as a chemolitoautotroph oxidizing hydrogen aerobically (Gomila *et al*., 2008). Recently, strains of the same species have been isolated in the United States as human pathogens from an abdominal abscess (strain 9-9768; Cárdenas *et al*., 2014) and from a brain abscess (strain CLUC; Greninger *et a*l., 2015). *M. llatzerense* has also been detected by PCR in other clinical samples (Teixeira *et al*., 2013). This species can be considered the first chemolitoautorophic bacterium that can be pathogenic for humans.

Genome sequences from the type strain of the species and from strain 9-9768 have been obtained in our laboratory. Comparative genome analysis of the three strains mentioned above, together with other genomes of RGM species, will be presented. Genomic approaches, with the derived pan-genome analysis of these microorganisms, are expected to open new insights to better understand the adaptation to different ecological niches, including the human body.

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10 (S)

"Genomics and Populations Genomics of the Legume-Rhizobial Symbiosis"

Beatriz Jorrín[1] and Juan Imperial[1,2]
E-mail: beatriz.jorrin@upm.es. 1. Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Madrid, Spain. 2. Consejo Superior de Investigaciones Científicas (CSIC), Spain

The establishment and maintenance of the symbiotic partnership results from a molecular conversation that guarantees specificity and guides the co-development of both organisms during root nodule formation. *Rhizobium leguminosarum* bv. viciae is a member of the α-Proteobacteria that can establish effective symbioses with members of the Fabeae legume tribe (*Pisum, Lathryrus, Lens* and *Vicia*). Previous studies have suggested that, although all *R. leguminosarum* bv. viciae isolates can effectively nodulate all Fabeae, different Fabeae select specific genotypes of rhizobia from those available in soil.

The aim of the present work was to characterize the genomic, genetic and molecular bases of the selection of specific *R. leguminosarum* bv. viciae genotypes by different host legumes (*Pisum sativum*, *Lens culinaris*, *Vicia faba* and *V. sativa*) from a well-characterized agricultural soil.

We established a population genomics methodology based on pooled DNA samples (Pool-Seq) from *R. leguminosarum* isolates obtained from different sources: legume plant hosts used as rhizobial traps (*P. sativum*, *L. culinaris*, *V. sativa* and *V. faba*), as well as the isolation of *R. leguminosarum* directly from soil. This approach allowed us to confirm the hypothesis that different plant hosts select specific subpopulations of rhizobia from the available population present in the soil.

We also set out to characterize the indigenous *R. leguminosarum* soil population avoiding plant selection, in order to compare it with previously characterized host-selected subpopulations from this soil. As a side result, we uncovered a large number of previously uncharacterized, non-symbiotic rhizobia that contribute to the population pangenome. Host preference for specific genotypes was especially relevant in the case of pea plants. They selected a *R. leguminosarum* population significantly different from that present in the soil. Quite on the contrary, lentil and fava bean plants did not show a significant genotype selection, and their nodule rhizobial populations reflected that present in soil (after one life cycle). Vetch plants revealed a certain genotypic preference, but not as substantial or as important as that from pea plants.

Given that plants can differentially select rhizobial genotypes and that viable rhizobia of those genotypes are released into soil after nodule senescence, we hypothesized that, in natural conditions after numerous cycles of selection-release, most nodules would be occupied by the preferred genotype/s in each plant. We experimentally tested this hypothesis in a mesocosm experiment aimed at mimicking these field conditions. We were able to demonstrate that the different plant hosts employed (*P. sativum*, *L. culinaris*, *V. faba* and *V. sativa*) selected different genotypes from those available in the P1 soil, and that the basis of selection was different for different plants. Pea and fava bean plants strongly selected for specific genotypes, but in different ways. Pea nodules were colonized by strains endowed with a large set of genes probably implicated in rhizospheric fitness, irrespective of the symbiotic genotype they harboured. This suggestion should be confirmed by *in situ* transcriptomic studies. Fava bean plants restricted their selection to a specific symbiotic genotype that was not always localized in the same symbiotic plasmid, or within the same chromosomal background. No hard conclusions could be obtained for vetch, although we suggest that this plant might behave dually, either as a selective host if a given genotype results in a rhizospheric advantage (such as in vetch$_B$ subpopulation), or as a non-selective host, with its nodules reflecting the genotypic diversity present in soil (such as in vetch$_A$ subpopulation). This last case was the situation found for lentils; none of the three subpopulations isolated from lentil nodules (initial lentil, lentil$_A$ and lentil$_B$) differed significantly, either among themselves, regardless of the number of plant selection cycles, or with respect to the initial soil population.

11 (S)

"Sequence analysis *Staphylococcus* spp strains Biofilm forming Bap positive"

Mercedes Cervera[1], Katerina Guzman[1], Miglè Ziemytè[1], Jose R Penades[2] y Mª Ángeles Tormo-Mas[1].
E-mail: tormo_man@iislafe.es. [1] Grupo de Infección Grave. Instituto de Investigación Sanitaria la Fe. Valencia. [2] Infection Immunity Inflamm, Glasgow Biomedical Research Cent. Glasgow. UK

Coagulase-Negative Staphylococci (CNS) is a group of adaptable and opportunistic pathogens whose ability to persist and multiply in a variety of environments causes a wide spectrum of disease in both human and animals. The *bap* gene, carried in a putative composite transposon, codes for bap, a multidomain protein with architecture characteristic of surface-associated protein and it is related with biofilm formation. Biofilm facilitates the adherence and colonization of Staphylococci, contributing to the evasion of the immunological defense and resulting in persistent infections (Cucarella C *et al*., 2004).

In this study, we sequenced and analyzed the whole genomes of two Bap positive high biofilm formatting strains, *Staphylococcus epidermidis* C533 and *Staphylococcus chromogenes* C483. Both of the strains were isolated from infected mammary glands of cows (Tormo MA *et al*., 2005). In previous studies it was observed that CNS are the most common pathogens associated with intramammary infections in dairy farms and that is the main reason why we are interested to know possible mechanisms of infection.

After analysis of the sequence, it was observed that the bap gene is located in a transposon however it was not in a pathogenicity island like *bap* gene of SaPIbov2 (Ubeda C *et al*., 2003).

Furthermore, the presence of mobile genetic elements like pathogenicity islands and phages were studied because they are very important in the virulence of *Staphylococcus*. *S. epidermidis* C533 has two islands and any phages and *S. chromogenes* C483 has only one pathogenicity island. All sequenced pathogenicity islands have their own typical structure, which includes integrase, repressor, excisionase, primase and terminase. Also we have found some genes like sigma 70, superantigens or *parB* within the islands.

Our following work will focus on study the function of some interesting genes found in the islands and to look for more possible virulence factors in the chromosome.

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12 (S)

"Double anticaries effect of the bacterium *Streptococcus dentisani*"

Arantxa López López

E-mail: lopez_aralop@gva.es. FISABIO. FISABIO. Fundación para el Fomento de la Investigación Sanitaria y Biomédica. Laboratorio de Microbioma Oral. Avda. de Catalunya, 21 / 46020 Valencia, España

Oral diseases, including dental caries and periodontitis, are among the most prevalent diseases worldwide and develop as a consequence of a microbial disbiosis. Several bacterial strains are being tested as potential oral health-promoting organisms, but usually they are species isolated from niches other than the site where they must exert its probiotic action, typically from fecal samples. We hypothesize that oral inhabitants associated to health conditions will be more effective than traditional, gut-associated probiotic species in key aspects such as colonization of the oral site where disease takes place or the possession of oral health promoting functions. As an example of these *active colonizers*, we describe the case of *Streptococcus dentisani*, a streptococcal species isolated from dental plaque of a caries-free individual. This species has a double probiotic action, as it inhibits the growth of major oral pathogens through the production of bacteriocins, and also buffers acidic pH (the primary cause of dental caries) through an arginolytic pathway. We propose the use of *S. dentisani* as a promising probiotic against tooth decay.

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"The Early Events Underlying Genome Evolution in a Localized *Sinorhizobium meliloti* Population"

Nicolás Toro, Francisco Martínez-Abarca and Manuel Fernández-López
E-mail: nicolas.toro@eez.csic.es. Grupo de Ecología Genética, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Calle Profesor Albareda 1, 18008 Granada, Spain

Population genetic analyses based on genome-wide sequencing data have been carried out for *Sinorhizobium medicae* and *S. meliloti,* two closely related bacterial species forming nitrogen-fixing symbioses with plants of the genus *Medicago*. However, genome coverage was low or the isolates had a broad geographic distribution, making it difficult to interpret the estimated diversity and to unravel the early events underlying population genetic variations and ecological differentiation. Here, we carried out genome-wide sequencing of a localized *S. meliloti* population with a well-defined structure and a high degree of coverage, to gain insight into the early forces driving its evolution. We first used Illumina paired-end reads technology to sequence a new clone of *S. meliloti* strain GR4, a highly competitive bacteria for alfalfa nodulation. The Illumina data and the GR4 genome sequence previously obtained with 454 technology were used to generate a high-quality reference genome sequence. We then used Illumina technology to sequence the genomes of 13 *S. meliloti* isolates representative of the genomic variation within the GR4-type population and present at different frequencies in root nodules. The genome sequences obtained were analyzed to determine nucleotide diversity, divergence times, polymorphism and genomic variation. Similar low levels of nucleotide diversity were observed for the chromosome, pSymB and pSymA replicons, and our findings suggest that this population may have experienced a process of demographic expansion. The isolates displayed other types of variation, such as indels, recombination events, genomic island excision and the transposition of mobile elements. The GR4-type population behaves as a stable genotypic cluster of genome-wide similarity, with most of the genome following a clonal pattern of evolution, probably arising due to and maintained by microhabitat separation, low levels of gene flow and plant host selection.

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"Analysis of the rhizospheric microbial communities of *Quercus pyrenaica* along an altitudinal gradient"

J.F.[1] Cobo-Díaz, A.J.[1] Fernández-González, P.J.[1] Villadas, N.[1] Toro, S.G.[2] Tringe, M[1] Fernández-López
E-mail: manuel.fernandez@eez.csic.es. *[1]Grupo de Ecología Genética, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Calle Profesor Albareda 1, 18008 Granada, Spain.[2]DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA.*

Functional and taxonomic rhizospheric microbial diversity for different states of a particular tree species forest is poorly understood. We used shotgun sequencing to analyze the microbial community composition in melojo-oak (*Quercus pyrenaica* Willd.) rhizospheric soil for three different situations along an altitudinal gradient: a) a low altitude, non-optimal site for forest maintenance; b) an intermediate altitude, optimal site for forest; and c) a high altitude, expansion site but without a real forest. We observed that, at each altitude, the same microbial taxa appear both in the taxonomic analysis of the whole metagenome and in the functional analysis of the methane, sulfur and nitrogen metabolism. Although there were no major differences at the functional level, there were significant differences at the phylogenetic level between the rhizospheres of forest and the highest, expansion site. Proteobacteria and Actinobacteria were the most differentially abundant phyla in forest soils compared to the expansion site rhizhosphere. Moreover, phyla Verrucomicrobia, Bacteroidetes and Nitrospirae were more highly represented in non-forest rhizosphere. Our study shows that differences of the rhizospheric microbial communities of the same tree species are due to relative abundance of each taxon. Moreover, these differences are indirectly dependent on the existence of forest canopy and altitude, which modifies the soil biogeochemistry.

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"Closed Genome *vs* draft Genome. A strategy to solve the location and ambiguities (SNPs) of repeated elements based on NGS genomic data"

Francisco Martínez Abarca.
E-mail: fmabarca@eez.csic.es. Grupo de Ecología Genética, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Calle Profesor Albareda 1, 18008 Granada, Spain

The continued improvements of Next Generation Sequencing technologies and protocols have opened an era where microbial genomes can be easily sequenced. However, the Impact of these NGS platforms have implemented new Genome Project Standard Categories to distinguish from very poor quality genomes to high quality closed genomes [1]. Based in these new NGS data, genome assemblies often resulted fragmented due to shorter read length, repeated regions and limitation in library construction protocols. In consequence while genome draft assemblies can be obtained in hours/days, finishing remained elusive and frequently take months and in some cases the effort can only be justified for high priority genomes [1].

Sinorhizobium bacteria are the microsymbionts of Medicago (e.g., *Medicago sativa* and *Medicago truncatula*), Melilotus, and Trigonella legume species. *S. meliloti* 1021 was the first sequenced strain within this genus, and is considered one of the Rhizobia reference genomes [2]. The genome consists of a single circular chromosome (~3.65 Mb) plus two large symbiotic (sym) plasmids of ~1.3 (megaplasmids) and ~1.6 Mb (chromids) in size. As occurs in other bacterial groups only a minor proportion of isolates belonging to *S. meliloti* species have been completely sequenced (as Standard Category).

We present a general strategy for the achievement of the complete genome of novel *S. meliloti* strains [3,4]. Little investments in terms of time, computational effort and lab work have been performed for finishing several multipartite microbial genome projects. The application of this strategy should increase the proportion of high quality closed genomes in databases.

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"Unraveling the universe of small RNA regulators in the legume symbiont *Sinorhizobium meliloti*"

José I. Jiménez-Zurdo and Marta Robledo
E-mail: joseignacio.jimenez@eez.csic.es. Grupo de Ecología Genética de la Rizosfera, Estación Experimental del Zaidín, CSIC.

High-throughput transcriptome profiling (RNAseq) has uncovered large and heterogeneous populations of small noncoding RNA species (sRNAs) with potential regulatory roles in bacteria. These sRNAs act mostly by protein-assisted base-pairing with target mRNAs to fine-tune post-transcriptional reprogramming of gene expression underlying bacterial responses to changing environments. Riboregulation impacts virtually any physiological process, including virulence of pathogenic bacteria. Here, we review our incipient knowledge on the structure and function of the noncoding transcriptome of the α-rhizobia *Sinorhizobium meliloti*, the nitrogen-fixing symbiotic partner of alfalfa and related medics. Several RNAseq-based surveys in *S. meliloti* have shown abundant transcription from hitherto regarded as noncoding intergenic regions (IGRs), strikingly high numbers of mRNA-derived RNAs and pervasive antisense transcription of protein-coding genes. sRNAs encoded within IGRs constitute the most extensively studied group of bacterial riboregulators. They are differentially expressed and modulate translation and/or stability of *trans*-encoded target mRNAs by short antisense interactions that, in enteric model bacteria, are facilitated by the RNA chaperone Hfq. The *trans*-sRNAs AbcR1 and AbcR2 are examples of Hfq-dependent sRNAs whereas EcpR1 does not bind Hfq. We will provide insights into the transcriptional regulation and activity mechanisms of these sRNAs for the targeting and control of multiple mRNAs involved in nutrient uptake (AbcR1/2) and cell cycle progression (EcpR1). Insights into the function of Hfq and endoribonucleases assisting riboregulation will be also presented.

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"Amazing Diversity of Microbial Rhodopsins: their Structure and Function"

Valentín Gordeliy

E-mail: valentin.gordeliy@gmail.com. Institut de Biologie Structurale J.-P. Univ. Grenoble Alpes-CEA-CNRS.

Just a few microbial rhodopsin, light-driven membrane retinal proteins, all from archaea, were known in the XXth century. The famous bacteriorhodopsin (BR) from *Halobium salinarum* discovered at the beginning of the seventies was a target of the best research laboratories. Discovered a little bit later halorhodopsins and sensory rhodopsins completed the story. These studies advanced different fields of science and led to the development of new methods. However, despite considerable efforts a major puzzle of BR, the molecular mechanism of proton translocation against proton gradient is still unresolved. A new era came with the advent of metagenomics and thousands of new rhodopsins have been discovered since 2000 when first bacterial proteorhodopsin was found. One of the biggest events was the discovery of channel rhodopisns and later of light-driven sodium pump KR2, the 'forbidden' protein by the then dominating rhodopsin paradigm. In this talk we will describe structure and function of microbial rhodopsins and in an overview of microbial rhodopsin studies we will show amazing diversity of their function and explain how very minor differences in their structure provide such diversity.

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"Importance of horizontal gene transfer on the evolution of thermophiles"

Juan M. Gonzalez
E-mail: jmgrau@irnase.csic.es. IRNAS-CSIC. Avda. Reina Mercedes 10, 41012 Sevilla, Spain

Microorganisms have evolved through years by adapting their genomes to survive under specific conditions. Thermophiles are a group of microorganisms thriving under continuous stress and must adapt to environmental changes, incorporating novel genetic features and physiological capabilities to inhabit extreme environments. The transference of genetic material among distantly related phyla has been reported to play a major role in microbial evolution. In this study, we will present a couple of examples of thermophilic microorganisms with metabolic features apparently gained through horizontal gene transfer. These cases are *Caldanerobacter subterraneus* and *Fervidobacterium* spp. Three genomes of *Caldanaerobacter subterraneus* strains are available. These strains posses a gene cluster encoding carbon monoxide dehydrogenase and energy converting hydrogenase, which are key enzymes in the metabolism of CO utilization, that forms distinct clades as seen by phylogenetic reconstruction. Four genomes of *Fervidobacterium* species are available. In this genus, around 40 transposase genes were detected per genome. A large fraction of these transposase genes had as closest relatives sequences from different phyla suggesting the potential for active horizontal gene transfer events. The question on whether the transference of DNA between distantly related species is uni- or bi-directional remains to be answered. This study present some evidences on the importance of horizontal gene transfer in thermophiles and analyzes if these transference events represent a process for the benefit of only some species or if it is a mechanism that microorganisms are looking for to all gain from the physical interaction and potential sharing of genetic material.

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"Alignment-Free Clustering of Whole Genome Sequencing Data or Assembled Draft Genomes"

Rosario San Millan[1]; Ilargi Martinez-Ballesteros[2]; Javier Garaizar[2]; Joseba Bikandi[2]
E-mail: joseba.bikandi@ehu.eus. [1]University of the Basque Country (EHU/UPV). Faculty of Medicine and Odontology.; [2]University of the Basque Country (EHU/UPV). Faculty of Pharmacy.

Within a couple of years, whole genome sequencing (WGS) will modify epidemiological methodologies in some countries. Rapid substitution is expected for pulsed-field gel electrophoresis, the gold standard for many microorganisms, and for other polymerase chain reaction- and sequencing-based methods used extensively for other pathogens. To handle WGS data, databases are being developed by the US Centers for Disease Control and National Center for Biotechnology Information, Public Health England, Canada's Public Health Agency, and others. Although those platforms use many approaches, they focus mostly on developing core genome multilocus sequence typing (MLST) or whole genome MLST. Furthermore, the amount of genes of specific species used in those techniques is not fixed but changes from platform to platform, and the computation requirements to operate them are huge. By contrast, alignment-free genetic sequence comparison methods are based on statistical analysis from k-mer frequencies and are applicable to WGS readings and, more often, assembled contigs and genomes. In this presentation, we describe a k-mer–based comparison method that we have developed for octanucleotides, recent improvements applied to accelerate computing, and its application in different situations. We show the extent to which our method agrees with other alignment-based methods and how sequence quality influences the results, as well as the incorrect classification at the species or serotype level of some genomes included in international databases. Part of the presentation focuses on the k-mer-based clustering of Salmonella, a pathogen studied in an international project to develop a platform involving MLST-based approaches for integrating genomics in the surveillance of foodborne pathogens.

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"'No plant is an island:' The sugarcane microbiome"

Juan Imperial
E-mail: juan.imperial@upm.es. Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Madrid, Spain. Consejo Superior de Investigaciones Científicas (CSIC), Spain

Sugarcane is a remarkable crop. A very efficient C_4-plant, it can continuously yield in excess of 150 Tm biomass / Ha / season under field conditions, which makes it the world's top producer. Although traditionally used for sugar production (and the ethanol derived from its fermentation), current research efforts are directed towards optimizing the use of this biomass to for second-generation biofuel production.
These high yields, especially in some areas, such as fields in Southeastern Brazil, are obtained with little fertilization and with no sign of progressively decaying yields. After the initial observations carried out by Brazilian microbiologist Joanna Döbereiner and the continued work of her team and former associates, it is widely accepted that the plant benefits from bacterial nitrogen fixation. A large number of remarkable sugarcane endophytes, some of them with the ability to fix nitrogen, have been isolated and characterized. However, their role in sugarcane nutrition has not been unequivocally substantiated. Having in mind that the intensive sugarcane cropping regime should impose a burden not only on the plant N economy but also on the supply of other nutrients, and that sugarcane-associated microorganisms could play a role in alleviating these burdens, we undertook the characterization of the sugarcane microbiome, along its development and in its different organs. Here we describe a comprehensive inventory of the structure and assemblage of the bacterial and fungal communities associated with sugarcane. Our analysis identified 23,811 bacterial OTUs and an unexpected 11,727 fungal OTUs inhabiting the endosphere and exosphere of roots, shoots and leaves. These communities originate primarily from the native soil around plants and colonize plant organs in distinct patterns. We identified core bacterial and fungal communities composed of less than 20% of the total microbial richness but accounting for over 90% of the total microbial relative abundance. The roots showed 89 core bacterial families, 19 of which accounted for 44% of the total relative abundance. Stalks are dominated by a diverse group of yeasts that represent over 12% of total relative abundance. The core microbiome described here comprise groups whose biological role underlies important traits in plant growth and fermentative processes that will be presented.

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