TITLE: Recognition of oral and gut microbes by specific antibodies

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Recognition of microorganisms by antibodies is a vital component of the human immune response. However, there is currently very limited understanding of immune recognition under health and disease, especially of a big portion of the human microbiome which is made up of as yet un-cultured bacteria. We have combined the use of flow cytometry and high throughput sequencing to describe the microbial composition of human samples, and its interaction with the immune system. We show the power of the technique in human faecal, saliva, oral biofilm and breast milk samples, labeled with fluorescent anti-IgG, anti-IgA or anti-IgM antibodies. Using Fluorescence-Activated Cell Sorting (FACS), bacterial cells were separated depending on whether they are coated or not with antibodies. Each bacterial population was then PCR-amplified and sequenced, characterizing the microorganisms which evade the immune system and those which were recognized by each immunoglobulin. We have applied this technique to study dental caries (tooth decay), showing that caries-free individuals have a significantly higher percentage of Ig-coated bacteria than caries-active individuals. In addition, we have identified a microbial-IgA recognition pattern that may serve as a biomarker of asthma years before the appearance of clinical symptoms in children. The application of the technique to other diseases may unravel the contribution of the immune response to microbial infections and polymicrobial diseases.

TITLE Comparative genomics of non-tuberculous mycobacteria: ecological and clinical implications

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The species classified as non-tuberculous mycobacteria (NTM), or rapid growing mycobacteria (RGM), are widely distributed in the environment and some of them are considered as emerging opportunistic pathogens. Nosocomial infections caused by NTM are usually difficult to treat due to their resistance to antibiotics or other external factors. The next-generation sequencing (NGS) technologies are opening new frontiers to different fields, and clinical microbiology is not an exception. The main objectives of this work imply the genome sequencing of NTM isolates (especially type strains), the identification of gene families, functional characterization, comparative analysis applying several clustering algorithms and the description of the core-pangenome of NTM. Briefly, the results obtained showed different rates of genome evolution and exclusive genes for each species (pangenes). Interestingly, the pangenome analysis of NTM has revealed also the presence of toxin-antitoxins (TA) systems in several of the strains compared. Curiously, most of the TA systems discovered in NTM are also present in *M. tuberculosis*. Furthermore, a brand-new TA system has been discoverd. The toxic potential of the proposed toxins and its neutralization with the hypothetical antitoxins have been tested *in vitro*.

Altogether, contributes to improve the NTM evolution knowledge, as well as to gain a better understanding of the mechanisms underlying their ability to adapt to different ecological niches; i.e., the resistome, the toxin-antitoxin systems or their ability to form biofilms. All these aspects affect the human's lifestyle. Definitively, the new NGS based information may lead to important breakthroughs, both in biotechnology and clinical microbiology.

TITLE: Evolution of vibrios in natural environment: lessons from a metagenomic study of skin mucus of the eel (*Anguilla anguilla*)

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Brief biography. Miguel Carda is Doctor in Biotechnology by the University of Valencia (Spain) (2017) and, currently, post-doctoral researcher at the University of Valencia (Spain). His research has been focused on metagenomic, genomic and molecular biology. He has been formed at the laboratories of F. Rodríguez Valera (Alicante), C. Amaro (Valencia) and M. Waldor Harvard (USA). Carmen Amaro is Full Professor of Microbiology at the University of Valencia (Spain). She is the leader of a research group devoted to the study of aquatic pathogens and its impact on Aquaculture. She has mainly focused her research on *Vibrio vulnificus*, a zoonotic pathogen, able to cause death by sepsis in humans and different species of aquatic animals. She has directed more than 20 research projects, has published more than 100 international papers on this matter, all of them in journals of Q1, and has a global H index of 32. Her research received a national award from the Spanish Ministry of Agriculture and Fisheries in 2001.

The hypothesis underlying this work was that the fish's skin-mucosal-surface (SMS) could be an adequate niche for the emergence of accidental human pathogens that cause intestinal diseases. In order to test this, we compared the SMS-microbiome of eels (*Anguilla anguilla*) to that of the surrounding water (W-microbiome). The eel was selected because it is euryhaline, lacks macroscopic scales and is covered by a dense layer of mucus. We also compared SMS-microbiome from eels sampled in different ecosystems, four natural water-bodies (salinity from less than 0.1 to 1 %) and one artificial (an intensive eel farm that uses water of

0.3% salinity). In parallel, we isolated selected bacterial species and compared their genomes with those in databases.

Gammaproteobacteria were the most abundant in SMS-microbiome regardless of eel origin (wild or farm), although the composition in genera and species was variable. Remarkably, potentially pathogenic *Vibrio* monopolized wild-eel's SMS-microbiome from natural ecosystems, *V. anguillarum/V. vulnificus* and *V. cholerae/V. metoecu*s being the most abundant ones in SMS from estuary and lake, respectively. Functionalities of the SMS-microbiome differed significantly from those of W-microbiome and allowed us to predict that successful colonizers contain specific genes for i) attachment (mainly by forming biofilms), ii) bacterial competence and communication, and iii) resistance to mucosal innate immunity, predators (amoeba) and heavy metals/drugs. In addition, we found several mobile genetic elements (mainly Integrative Conjugative Elements) as well as a series of evidences suggesting that bacteria exchange DNA in SMS. Finally, the genome of a *V. metoecus* strain isolated from SMS was compared with all published genomes of the same species. The SMS isolate presented characteristics intermediate between *V. metoecus* from water/extra-intestinal infections and *V. cholerae* O1 from cholera patients suggesting that HGT events between close *Vibrio* species could take place in this mucosal environment.

In conclusion, we have obtained metagenomic and genomic evidence in favor of the hypothesis on the role of fish mucosal surfaces acting as driving forces for selecting the best adapted bacteria to colonize and persist in mucosal surfaces e.g. the human intestine.

TITLE: Crossing lines: Viruses encode ribosomal proteins

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Interactions between viruses and cells have played a major role in the evolution of life. In particular, viruses have a high impact on the composition of microbial communities due to their abundance and profound significance as agents of microbial mortality. Numerous studies have previous shown that viruses and their hosts are engaged in bilateral horizontal gene transfer which have significant consequences for the mode of virus-host interaction. Viruses infecting marine cyanobacteria present one of the most illustrious examples. These viruses are known to carry their own genes encoding some of the key components of photosynthesis. It has been also shown that these host-derived genes are translated and the produced proteins boost the energy metabolism in the infected cell, thereby increasing viral fitness. More recently, metagenomic studies have also contributed to expanding the repertoire of such metabolic host-derived genes found in viruses. Among the most unexpected discoveries was the presence of a high number of ribosomal proteins-encoding genes within bona fide viromes. Ribosomal proteins interact with the rRNA to form the ribosome, the molecular machinery for protein biosynthesis, and are the hallmark of cellular life forms. Here we explored the presence of ribosomal proteins within publicly available viral genomes. Fifteen viral genomes (14 bacteriophages and one eukaryotic virus) encoding ribosomal protein homologues were identified. This was surprising because even giant viruses of protists, such as mimiviruses and pandoraviruses, lack such genes in their genomes. Five different ribosomal proteins were found to be encoded by viruses, although the functional importance of these acquisitions is still speculative. Our results break the long-standing dogma that viruses do not encode ribosomal proteins and show that genes from virtually all functional categories are subject to horizontal transfer.

TITLE: Molecular insights into factors controlling marine picocyanobacterial diversity and abundance

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Marine picocyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are numerically dominant in vast tracts of the ocean. Using a combination of radiotracer and flow cytometric sorting studies we have evaluated the importance of each genera in marine CO_2 fixation [1]. Although a minor component numerically, photosynthetic picoeukaryotes (PPEs) are also important marine primary producers. However, these PPEs can also act as bacterivores in oligotrophic gyre ecosystems, actively consuming picocyanobacteria [2, 3]. Such biotic control of natural picocyanobacterial populations likely also includes viral lysis. Indeed, cyanophages actively shut down host CO_2 fixation whilst maintaining photosynthetic electron transport during infection [4].

As well as biotic control of cell abundance, natural picocyanobacterial populations are also structured genetically by abiotic factors such as temperature, nutrient availability and light intensity [5]. Specifically for *Synechococcus*, we have developed a high resolution phylogenetic framework to fine tune the geographical partitioning of this genus *in situ,* which is particularly well typified along Atlantic Meridional Transects [6]. In concert with in-depth genomic and metagenomic studies, we aim to uncover the specific adaptation mechanisms of the numerous *Synechococcus* phylotypes observed, that will help explain the successful colonization of this genus throughout the marine environment.

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ENVIRONMENTAL VIBRIOS: "A WALK ON THE WILD SIDE"

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Climate change has caused a worldwide increase in reports of vibrio-associated diseases with ecosystem-wide impacts on humans and marine animals. In addition, the rapid growth of aquaculture has been the source of anthropogenic changes on a massive scale. Animals have been displaced from their natural environments, farmed at high densities and exposed to environmental stresses, including antibiotic treatment. Unfortunately but not surprisingly, marine farming areas constitute ideal locations for the study of the emergence of pathogens in real time. The vast majority of our knowledge on vibrio pathogenesis is based on the ancient and well-studied human pathogen, *V. cholerae.* Often neglected and relegated to specialized journals are vibrio infections in non-human species. While the studies of animal pathogens have benefited from the era of genomics, the search for pathogenesis determinants is often biased by what is known from human pathogens, precluding the discovery of new mechanisms specific to marine animal species. Here I describe why vibrios from the wild (as opposed to laboratory model strains) are pertinent to address basic questions such as evolutionary and ecological dynamics of pathogens, as well as how they are a source of original molecular mechanisms for virulence, cell to cell interaction and genetic regulation.

TITLE Genomic and physiological analysis of soil ammonia oxidising archaea: always good, never bad, sometimes ugly

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Ammonia oxidation is the first and rate-limiting step of nitrification. This microbially-mediated process transforms ammonia to nitrate, the most reduced and oxidized forms of nitrogen, respectively. While an essential component of the global nitrogen biogeochemical cycle, this process also has major economic and environmental consequences, leading to an annual loss of >$15 billion of fertilizer, nitrate pollution of water and the generation of the greenhouse gas nitrous oxide. For over a century this process was thought to be dominated by ammonia oxidizing bacteria (AOB). However, just over a decade ago, ammonia oxidizing archaea (AOA) were discovered and found to be globally ubiquitous and abundant in soils and other environments, and considerable research effort has gone into understanding whether these organisms are truly functionally analogous to their bacterial counterparts. While most initial studies used measurements of abundance and correlation analyses to imply functional attributes, the use of cultivation, genomics and incubation studies have provided crucial insights into their unique contribution to soil nitrogen cycling. Specifically, genomic and physiological characterization of two novel soil AOA, *Ca.* Nitrosotalea devanaterra and *Ca.* Nitrososcosmicus franklandus (isolated from acidic and neutral pH soil, respectively) have indicated that while AOA are central to ammonia oxidation processes in many soils, fundamental aspects of their physiology indicate that their contributions to deleterious environmental processes are much less than that of AOB in terrestrial systems.

TITLE The οικοσ of marine bacteria

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Environment selects for the organisms that are present; but what are the environmental properties that describe the bacterial *οικοσ* – oîkos or house – the word from which ecology derives? Longhurst *et al.* made a very useful characterisation of marine provinces, which is helpful as a broad-brush approach to describing the conditions that plankton experience in any part of the global ocean. Yet, useful as they are, the Longhurst provinces do not consider space scales that are of primary relevance to marine bacteria – *i.e.* micrometres to millimetre scales.

At these dimensions, Reynolds number is very low and, to our anthropocentric view of the world, everything is very weird. Viscous forces dominate, which means for example that moving bacteria have no inertia. If a flagellum stops beating, the cell moves only a few angstroms before coming to a complete standstill. Physical and chemical properties of water at the microbial scale have implications for bacterial evolution, particularly in the oligotrophic oceans. Here, nutrient concentrations are very low – ammonium concentrations are typically ca. 10nmol L^{-1} – and microbes are widely spaced from each other. Although large numbers of bacteria are present (>10^5 mL^{-1}), because they are so small the distance to the next cell is many hundreds of cell diameters. The oceans are mostly empty space.

The bacteria that dominate in the oligotrophic ocean gyres (e.g. *Prochlorococcus,* and *Pelagibacter ubique*) are small cells and have streamlined genomes. It can be argued that streamlined genomes either reflect, or are, a consequence of the environment. For example, *Prochlorococcus,* and *Pelagibacter* are both non-motile. Why should this be? It is intuitive to think that, when nutrients are in short supply, it would be beneficial to swim to where nutrient concentrations are higher. However, at low Reynolds number, swimming merely drags with the cell as it moves any zone of depletion around it; it does not escape by moving, the consequences of depleting nutrients in its immediate environment. However, rates of molecular diffusion are extremely rapid and mean that there would be no advantage to a cell to be motile in the oligotrophic ocean.

Does thinking about the physical/chemical properties at spatial/temporal scales of relevance to bacteria, help to understand the process of genome streamlining? What predictions could be made about the evolutionary advantage for a bacterium to retain certain functions in the oligotrophic ocean, particularly in the context of life at low Reynolds number?

TITLE: Hypersaline systems as natural laboratories to study virus-host interactions

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Hypersaline environments constitute a privileged scenario to study virus-host interactions in natural settings since they are mainly inhabited by a relatively low number of prokaryotic species and their viruses. In fact, they present the highest concentrations of virus-like particles (VLPs) reported so far for aquatic systems, with concentrations as high as 10^{10} VLP/ml of water. Viruses are frequently the main biological factor controlling their host's communities since bacterivory normally disappears over 25% of total salts.

Here we present examples of how hypersaline waters can be manipulated to address relevant aspects of virus-host interactions. We will focus on the study of viruses infecting the cosmopolitan hyperhalophilic Bacteroidetes *Salinibacter ruber*. Previous results indicate that *Salinibacter*-related viruses are very active in crystallizer waters, which could be partially explaining why *S. ruber* is generally outnumbered by haloarchaea in spite of the fact that it is as halophilic as the most halophilic archaeon.

In the first example, *S. ruber* viruses were investigated in a mesocosmos experiment in which a natural virus assemblage was challenged with a mixture of *S. ruber* strains. Only viruses closely related to previously isolated viruses (strictly lytic and with wide host range) were enriched in spite of their low abundance in the natural sample before the incubation. Conversely, the most abundant *S. ruber* viruses in the original samples (closely related to very narrow range isolates) disappeared after the incubation with their hosts. This is consistent with a scenario in which host range, not only virus and host abundance, is a key factor in determining virus fate in nature.

In the second example, we scale this manipulation up to real world level by inoculating a *S. ruber* strain in a crystallizer pond from a solar saltern in Mallorca, Spain. The strain, isolated 15 years ago from this saltern, was not present in the pond at the moment of isolation. The rationale was providing the community with a new *winner* to monitor whether indeed viruses will be killing them, as expected by the current models, and how the introduction of a new host impacts the microbial community (virus included) as a whole.

The direction of gene transfer among pairs of genomes

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Microorganisms adapt rapidly to the environment and the exchange of DNA through events of horizontal gene transfer (HGT) represents a major mechanism for rapid evolution. This study focuses on evaluating the direction of some identified HGT events among pairs of microorganisms from different phyla. In this way, we can predict the origin and fate of DNA fragments which is of relevance to understand the evolutionary history of the microbial species. The pan-genome includes the known set of genes present within a taxon. The core genome represents the set of common genes within that taxon. By evaluating pairs of microorganisms (generally at the genus level), we could separate those shared genes belonging to the core genome in a genus and to the pan-genome in the other. Among the genes that are found in representatives of the two genera being compared, the ones that belong to the core genome of one of the genera will be considered to be originated from this genus while those genes only detected in a minor fraction of the representatives of a genome (i.e., belonging to the pan-genome of the genus) will be considered to be acquired in this second genus. We have been able to compare several pairs of taxa and to deduce that most of the shared genes, putatively acquired by HGT, are present in most species of one of the compared genera and sparsely found in the other and viceversa with the rest of shared genes. Thus, we can relatively easily define the origin of the HGT events involving those genes and pairs of taxa. The procedure comparing the genome sequences available from two different taxa will provide with valuable information to deduce the evolutionary history of specific taxa, its relevance to functional capabilities within these taxa and will decisively contribute to our understanding of the interactive relationships among microorganisms in the natural environment.

TITLE The Sugarcane Microbiome: A Key Element for Its Sustainability

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Sugarcane (*Saccharum spp.*) is a complex hybrid cultivated in tropical and subtropical regions worldwide, mainly for its sugar, but also for its biomass, given that sugarcane boasts the highest primary productivities for any crop. Under optimal conditions, yields exceeding 150 Tm/Ha have been recorded, and the average for Brazil, the first world producer, exceeds 65 Tm/Ha over the *ca.* 10 million Ha planted.

Contrary to the situation encountered in many countries, sugarcane cultivated in South-Central Brazil has traditionally required little nitrogen fertilization. This led Döbereiner and collaborators to propose that a significant portion of sugarcane's N budget is provided by diazotrophic microorganisms in close association with sugarcane. Although sugarcane-associated nitrogen fixation has been proven in the field, and although diazotrophs have been isolated from sugarcane, the exact nature of the diazotrophs responsible for sugarcane nitrogen fixation remains elusive.

We have undertaken a thorough characterization of the sugarcane microbiome, across different organs and throughout the growing season. An unexpectedly large and diverse microbiome, made up of bacterial and fungal endophytes and exophytes has been uncovered. The microbiome is dominated by a core microbiome of few, very abundant OTUs. Many of those had not been previously described in sugarcane. A community-based culture collection of the most abundant isolates from the sugarcane microbiome has been established and characterized. This collection contains a large proportion of the OTUS constituting the core sugarcane microbiome, thus opening the way to microbiome reconstruction experiments. Preliminary tests suggest that many of the isolates exhibit desirable plant-growth promoting properties, either individually or in mixtures.

This work was supported by contracts from Repsol and Repsol-Sinopec.

TITLE : A new family of Staphylococcus aureus phages isolated from patients with cystic fibrosis.

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Mobile genetic elements (MGE) such as pathogenicity islands and bacteriophages are very common in clinical isolates of *Staphylococcus aureus*. Moreover, they are closely related with each other and its interaction leads to dissemination of various virulence factors. For example, bacteriophages are responsible for induction, packaging and transference of *Staphylococcus aureus* pathogenicity islands (SaPIs). As pulmonary infection with *S aureus* is a frequent problem in patients with cystic fibrosis we were interested in the role of MGE and its interaction in these strains.

During our study, we have tested 200 *S. aureus* isolates from 118 cystic fibrosis patients. We have analyzed their MGEs and we have observed that 77,5% of these strains contained bacteriophages with integrase type III, interestingly 85,4% of these contain a conserved packaging module that was different from previously described phages. In all of the cases phages contained TerL, endonuclease HNH and a hypothetical protein that might be a new TerS. We determined that deletion of this protein doesn't affect phage DNA replication but completely eliminated phage packaging and infectivity. As obvious morphological changes in phage structure were observed in this mutant, we want to demonstrate cos-site cleavage by this HNH-TerS-TerL complex. In future, we would like to characterize all proteins implicated in packaging machinery of these phages. Moreover, it is important know the implication of these phages in mobilization and transference of virulence factors, codified in other MGE like SaPIs. That might lead to better adaptation of these strains in cystic fibrosis patients with pulmonary infections.

TITLE: The rhizosphere microbiome of burned holm-oak: potential role of the genus *Arthrobacter* in the recovery of burned soils.

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After a forest wildfire, the microbial communities have a transient alteration in its composition. But the biological role of this microbial population up to the recovery of the ecosystem is not well established. Thus, it is necessary to understand the plant microbe interactions that occur in burned soils. We evaluated the microbial communities associated with holm-oaks 3 years after a forest fire by 454 and Illumina sequencing of rhizospheric DNA. We identified and examined the main taxa of soil bacteria in response to wildfire disturbance, emphasizing its isolation and characterization. Deep sequencing showed that the genus *Arthrobacter*, from the phylum Actinobacteria, was more than 21% of the total community; while the genus *Bradyrhizobium*, from the phylum Proteobacteria, was the most negatively affected by fire. 55 *Arthrobacter* strains were isolated and characterized using RAPDs and sequencing of 16S rDNA gene. Moreover, these strains were assayed in their PGPR traits and their interaction when used as inoculants in plants. Our results indicate that isolated *Arthrobacter* strains present a very high genetic diversity, and they could play an important ecological role in interaction with the host plant by enhancing aerial growth. Most of the selected strains exhibit in vitro a great ability to degrade organic polymers as well as possibly presenting a direct mechanism for plant growth promotion. All the above data suggests that *Arthrobacter* can be considered as an excellent PGPR that may play an important role in the recovery of burned forests.

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TITLE Is viral metagenomics missing something?: A tale of Single-Virus Genomics

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It is well known that cultivation techniques inefficiently capture naturally occurring viral diversity in nature. To circumvent these limitations, past and current efforts based on metagenomics have significantly and unquestionably broadened our knowledge on viral genomics in marine ecosystems. However, even with expeditions such as *Tara Oceans,* the available reference viral genomes – cultivated and uncultivated – fail to recruit most viral metagenomic reads. Thus, there is agreement that much viral diversity remains to be discovered in the oceans. Recently, single-cell genomics has emerged as a powerful strategy to disentangle the the genetic information of many abundant and ecologically significant prokaryotes in marine and other environments. In this study, we will show the results on the implementation and optimization of cutting-edge techniques based on single-cell genomics to uncultured marine viral assemblages from epi- meso- and bathypelagic samples collected in the Mediterranean Sea and South Atlantic. Our single-virus genomics data has enabled the discovery of some of the likely most abundant and ecologically relevant viral species at the global scale, such as vSAG 37-F6, which were overlooked by current methodologies. Finally, we will discuss why the genomes of these viral species have not been assembled by metagenomics. Our data point that microdiversity matters for metagenomic assembly.

PHYLOGENOMICS OF THE *PSEUDOMONAS SYRINGAE* SPECIES GROUP

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The genus *Pseudomonas* is taxonomically divided in two phylogenetic lineages (*P. aeruginosa* and *P. fluorescens*) based on multilocus sequence analysis (MLSA) of four housekeeping genes (16S rRNA, *gyrB*, *rpoB* and *rpoD*). *P. fluorescens* lineage is divided into six groups, one of them represented by *Pseudomonas syringae*. Currently, the *P. syringae* species group is subdivided into more than 60 pathovars defined by pathogenic characters, nine genomospecies defined by DDH and 13 phylogenetic groups (phylogroups) defined by MLSA.

With the objective to clarify the taxonomic species delineation in the *P. syringae* group, more than 100 strains of species within the group and whose genomes have been sequenced and are available in databases were analysed by a phylogenomic approach. MLSA, Average nucleotide identity based on BLAST (ANIb) and Mummer (ANIm), Genome-to-Genome Distance Calculator (GGDC) as well as core and pangenome analyses were performed to delineate genomic species. Genes encoding virulence factors, secretion systems and effectors, were studied in detail to clarify the pathogenicity potential of strains in the *P. syringae* group. Genome comparison of close-related pathogenic and non-pathogenic strains were also performed.

All methods tested were concordant and allowed to infer the taxonomic affiliation of all genomes analysed, some of them not correctly assigned to species. The pathovars did not follow the genomic clusters already defined. New genomic groups can be distinguished belonging to putative novel species. Genomic and phylogenetic approaches will provide the basis for a more reliable demarcation of *Pseudomonas* phytopathogenic species.

TITLE: Pangenome Evolution in the Marine Bacterium *Alteromonas*

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We have examined a collection of the free-living marine bacterium *Alteromonas* genomes with cores diverging in average nucleotide identities 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 1.4 Mb (ca. 30% of the typical strain genome size). Recombination rates along the core were high among strains belonging to the same species (37.7–83.7% of all nucleotide polymorphisms) but they decreased sharply between species (18.9–5.1%). 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 as they are found, with nearly identical sequences, in different species. Models for the generation of this genomic diversity involving phage predation are discussed.

STRUCTURE AND SPECIFICITY OF BACTERIOPHAGE RECEPTOR-BINDING PROTEINS

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Bacteriophages, the most numerous biological replicating entities in the world, are complicated one-time nano-machines that transfer their genomic material into susceptible host bacteria. They have specialized proteins for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction.

We have solved the detailed structures of several of these receptor-binding proteins and have shown them to be mainly beta-structured, but structurally highly diverse and containing several new protein folds. Structures of the RBPs of the coli-phages T4, T5 and T7, of the Salmonella phage epsilon15 and of the Staphylococcus phages S24-1 and K will be shown. Ongoing structural, mutational and binding analysis of RBPs with receptors and receptor analogues will be discussed.

Bacteriophage receptor-recognizing proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptor-binding domains may allow retargeting of phages to alternative host bacteria.

TITLE: Modern methods to determine which virus goes with which host

Microbes are recently recognized as driving the energy and nutrient transformations that fuel Earth's ecosystems in soils, oceans and humans. Where studied, viruses appear to modulate these microbial impacts in ways ranging from mortality and nutrient recycling to complete metabolic reprogramming during infection. As environmental virology strives to get a handle on the global virosphere (the diversity of viruses in nature) the next step is to link as many of these viruses to their natural hosts as possible. In this lecture, I will present the modern informatics and experimental methods available to do so.

NAME

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TITLE: Population genetics of 142 cyanophage genomes: Viruses can behave as 'species'

Microbes are recently recognized as driving the energy and nutrient transformations that fuel Earth's ecosystems in soils, oceans and humans. Where studied, viruses appear to modulate these microbial impacts in ways ranging from mortality and nutrient recycling to complete metabolic reprogramming during infection. As environmental virology strives to get a handle on the global virosphere (the diversity of viruses in nature) we are challenged to organize this 'sequence space'. In this lecture, I will share current thinking on how to establish viral taxonomy – at the species and genus level – through population genetic and network based approaches.

NAME

Ecogenomics of Microbes

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TITLE Diversification and coherence in a species of pelagic freshwater bacteria

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In many prokaryotic genera a clustered phylogeny is observed, comparably to the occurrence of species in sexually reproducing organisms. Yet, the diversity within bacterial species can be vast compared to what is known from macroorganisms, implying the coexistence of various closely related genotypes within a single habitat. Two major questions can be addressed. (i) What is the ecophysiological relevance of the diversity observed within lineages/species and (ii) what are the driving forces providing coherence within species?

Polynucleobacter is a particularly interesting taxon for studying these issues in freshwaters due to its cosmopolitan distribution and high global abundance. *Polynucleobacter asymbioticus*, prevalent in dystrophic ponds in the Austrian Alps, was selected for polyphasic investigations, including the targeted isolation of strains from different sites, genetic analysis (multi locus sequence typing and genome sequencing) in the context of geographic structure and physiological testing.

Overall, genetic variability occurs most notably in genomic islands, to some of which specific functions could be assigned and demonstrated in ecophysiological experiments. Interspecies genome comparisons provide evidence for recent exchanges of genomic islands across species boundaries. The variability resulting from the presence of different genomic islands might provide fine scale niche differentiation and thus, enable the coexistence of numerous closely related genotypes. On the other hand, analysis of the core genomes indicate high recombination rates between conspecific strains, not significantly reduced by the geographic separation of the respective habitats. This may indicate that homologous recombination is the main factor providing genetic coherence within the species.

TITLE: Mobilization mechanism of pathogenicity islands by enogenous phages in *Staphylococcus aureus*

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Staphylococcus aureus is a commensal bacteria and a human pathogen. As a pathogen, this bacterium is able to cause a variety of community and hospital acquired diseases, including skin abscess, food poisoning, sepsis or toxic shock syndrome. The broad range of infections caused by *S. aureus* are related to a number of virulence factors that allow it to adhere to the surface, invade immune system and cause harmful toxic effects to the host.

Most of these virulence factors are encoded in mobile genetic elements (MGE). During our study, we concentrate in *S. aureus* pathogenicity islands (SaPIs) which are maintained in the chromosome by a repressor (Stl). This repressor is activated by some helper phages, which encode specific, nonessential inductor proteins. Binding of repressor Stl and inductor induces the SaPI excision, replication and packaging cycle. These events allow transference of SaPIs spreading virulence factors among different bacteria.

In this study, we tested SaPIs mobilization of 15 sequenced clinical *S. aureus* strains. Seven strains were induced and mobilized by its endogenous phages. We focused in the regulation module of two strains whose SaPIs present the same Stl. We identified the endogenous phage responsible of SaPI induction and its specific inductor protein. This protein corresponds to a hypothetical protein with a conserved domain. Mutant of this protein was not able to induce and mobilize the SaPI. We also demonstrated the binding Stl-inductor and the existence of allelic variants of this protein codified in other phages with different affinities for Stl. This confirms that this mechanism is general for others strains. In the future we would like to characterize the protein structure, the binding motif to Stl and to determine the function in the phage.

Microdiversification in genome-streamlined ubiquitous freshwater Actinobacteria

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Actinobacteria of the acI lineage are the most abundant microbes in freshwater systems, but there are so far no pure cultures of these organisms, possibly due to metabolic dependencies on other microbes. This, in turn, has hampered an in-depth assessment of the genomic basis for their success in the environment. In my talk, I will present genomes from the first 16 axenic cultures of acI Actinobacteria. The isolates were not only of minute cell size, but also amongst the most streamlined free-living microbes, with extremely small genome sizes (1.2-1.4 Mbp) and low genomic GC content. Genome reduction in these bacteria has led to auxotrophy for various vitamins, amino acids, and reduced sulphur sources, thus lending support to the predictions of the 'Black Queen' hypothesis. Genome analysis, moreover, revealed a surprising degree of inter- and intraspecific diversity in metabolic pathways, especially of carbohydrate transport and metabolism, and mainly encoded in genomic islands. The striking genotype microdiversification of acI Actinobacteria might explain their global success in highly dynamic freshwater environments with complex seasonal patterns of allochthonous and autochthonous carbon sources. We propose a new order within Actinobacteria ('*Candidatus* Nanopelagicales') with two new genera ('*Candidatus* Nanopelagicus' and '*Candidatus* Planktophila') and nine new species.

The Reverse Transcriptases associated to the CRISPR-Cas Systems

Nicolás Toro*, Francisco Martínez-Abarca and Alejandro González-Delgado

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Prokaryotic genomes harbor a large number of diverse, uncharacterized reverse transcriptases (RTs) and RT-like sequences. Over 50% of these RTs correspond to proteins (IEP) encoded by group II introns, which are large catalytic RNAs and mobile retroelements (1). Distinct RTs surveys and phylogenetic analyses have shown RT-like sequences to be associated with CRISPR/*cas* elements, and some RTs are fused to *cas* genes. CRISPR and their *cas*-associated genes encode a sequence-specific mechanism of defense against bacteriophages and plasmids, consisting of an array of short repetitive sequences (≈40bp long) separated by equally short spacer sequences. The Cas1 and Cas2 proteins form a complex that represent the adaptation module which is required for the acquisition of DNA spacers into the CRISPR array. Recently, the CRISPR-*cas* loci have been classified into two clases, five types and 16 subtypes based on the analysis of signature protein families and features of the architecture of *cas* loci (2). Interestingly, it has been recently reported that one of this RT-Cas1 fusion enables the acquisition of RNA spacers *in vivo* in an RT-dependent manner, which raise the possibility of spacer acquisition involving reverse transcription potentially beneficial against parasitic RNA sequences or highly transcribed DNA phages and plasmids as well as other host-beneficial mechanism for gene expression regulation (3). Here, we compile data of RTs associated to CRISPR-CAS systems and analyzed their overall architecture to get insights into the phylogenetic relationships within these RTs and with this particular group of CRISPR/*cas* elements.

(1) Toro N, Nisa-Martínez R. (2014) PLoS One. 9 :e114083.
(2) Makarova *et al.* (2015) Nat Rev Microbiol. 13:722-736.
(3) Silas *et al.* (2016) Science. 351:aad4234.

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Cyanophage 'photosynthesis' with viral PSI & PSII proteins

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Cyanobacteria are important contributors to primary production in the open oceans. Over the past decade various photosynthesis-related genes have been found in viruses that infect cyanobacteria (cyanophages). While photosystem-II (PSII) genes are common in both cultured cyanophages and environmental samples, viral photosystem-I (PSI) genes have only been detected in environmental samples to date, via metagenomics. In my lecture I will describe a targeted strategy used to isolate cyanophages that carry PSI gene cassettes (and PSII genes). Furthermore, the influence of the phage encoded photosynthetic proteins on the host's photosynthesis will be discussed.

TITLE: Linking phylogenomic species concepts to functional traits in marine bacteria

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Paul Jensen received a BS in marine biology from the Florida Institute of Technology, an MS degree in microbiology from San Diego State University, and a PhD in marine biology from the Scripps Institution of Oceanography, University of California San Diego, where he is currently a professor at the Center for Marine Biotechnology and Biomedicine. His research interests lie at the interface of marine microbiology and natural product chemistry. His group addresses fundamental questions related to the diversity and distributions of bacteria in the marine environment while targeting taxa that produce biologically active secondary metabolites. The compounds produced are explored for useful applications and to assess the functional roles of secondary metabolites in marine systems. His studies employ molecular and culture-dependent techniques with an emphasis on sequence based approaches to investigate the ecology and evolution of secondary metabolism. The overall goals of his program are to ask which microbes produce natural products, where they live, and why they make them in the context of developing better methods for natural product discovery.

TITLE

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The enigmatic SAR202 cluster up close: shedding light on a globally distributed dark ocean lineage involved in sulfur cycling

The dark ocean microbiota represents the unknown majority in the global ocean waters. The SAR202 cluster belonging to the phylum Chloroflexi was the first microbial lineage discovered to specifically inhabit the aphotic realm, where they are abundant and globally distributed. The absence of SAR202 cultured representatives has been a significant bottleneck towards understanding their metabolic capacities and role in the marine environment. In this work, we use a combination of metagenome-assembled genomes from deep-sea datasets and publicly available single-cell genomes to construct a genomic perspective of SAR202 phylogeny, metabolism and biogeography. Our results suggest that SAR202 cluster members are medium sized, free-living cells with a heterotrophic lifestyle, broadly divided into two distinct clades. We present the first evidence of vertical stratification of these microbes along the meso- and bathypelagic ocean layers. Remarkably, two distinct species of SAR202 cluster were found to be highly abundant in nearly all deep bathypelagic metagenomic datasets available so far. Many SAR202 members appear to be sulfite-oxidizers and are predicted to play a major role in sulfur turnover in the dark water column and suggesting an unsuspected availability of these nutrient sources to allow for the high abundance of these microbes in the deep sea.

TITLE : From a Phylogenetic Tree to Discovery of Inward H+ pump Xenorhodopsin and Alternative Optogenetic Approach

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We will describe discovery of inward membrane protein light-driven proton pumps. Inward plasma membrane native proton pumps have been unknown. Generation of electrochemical proton gradient is the first and universal step of cell bioenergetics provided by outward proton pumps. Therefore, the existence of inward was not expected**.** In our talk we describe comprehensive functional studies of the representatives of the yet non-characterized xenorhodopsins from *Nanohaloarchaea* family of microbial rhodopsins. We showed in experiments that they are inward proton pumps. We proved that in model membrane systems, *E.coli* cells, human embryonic kidney cells, neuroblastoma cells and rat hippocampal neuronal cells. We also solved the structure of a xenorhodopsin from *Nanosalina* (*Ns*XeR) and suggest a mechanism of inward proton pumping. We demonstrated that the *Ns*XeR is a powerful pump which is able to elicit action potentials in rat hippocampal neuronal cells up to their maximal intrinsic firing frequency, proving that the inwardly directed proton pumps are suitable for light induced remote control of neurons and are an alternative to the well-known cation selective channelrhodopsins.