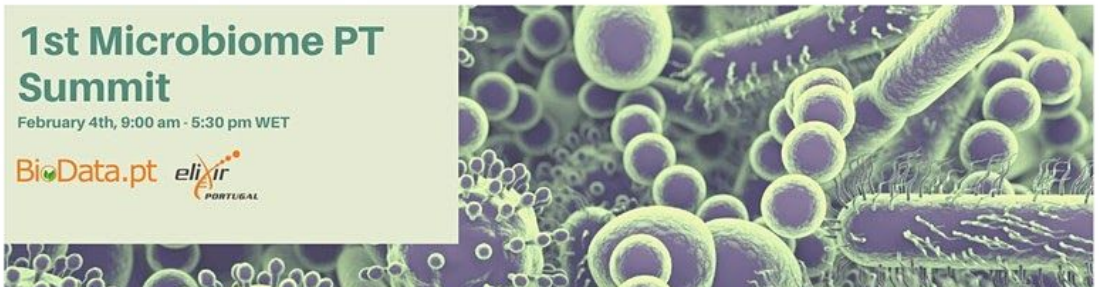

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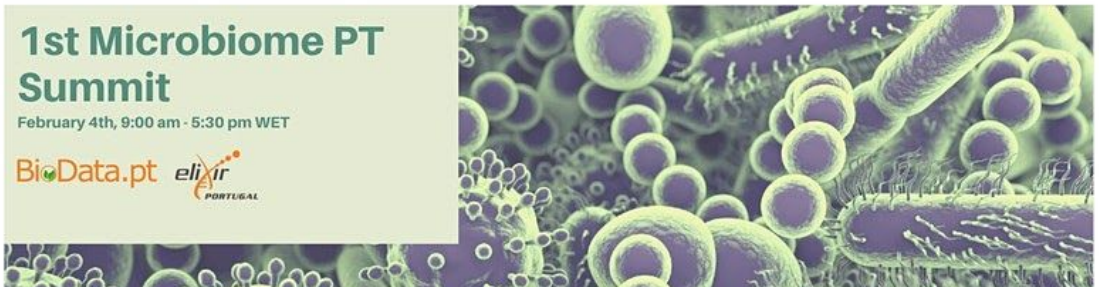

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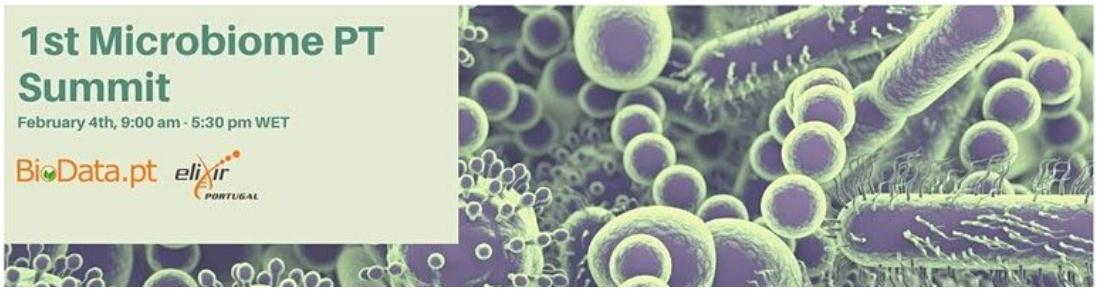


Microbiomes of humans and other mammals

1 - Dina Carpinteiro - Technician at Instituto Nacional de Saúde Dr. Ricardo Jorge

Contamination of low biomass samples with environmental strains may seriously impact results of microbiome studies

Dina Carpinteiro 1, Deodália Dias 2, Luís Vieira 1

1 Instituto Nacional de Saúde Doutor Ricardo Jorge, 2 Faculdade de Ciências da Universidade de Lisboa

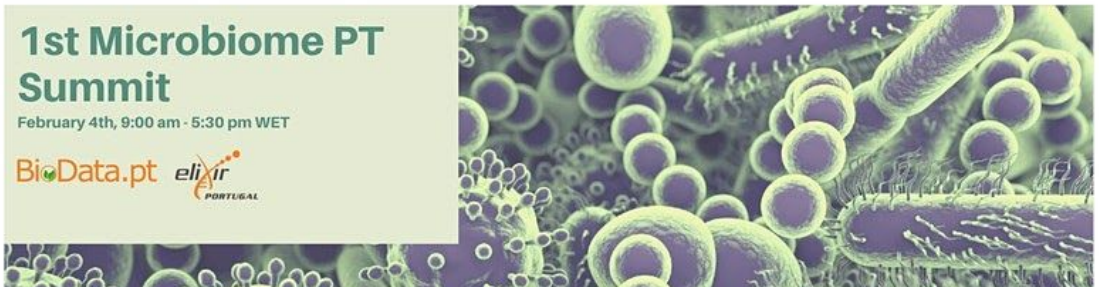
In the past decade human microbiome studies benefited significantly from advances in sequencing technologies. However, these studies also have drawbacks. These include the presence of a low microorganism biomass in some body parts, which leads to technical limitations in the detection of minority strains, and contamination with environmental strains during the collection and processing of samples, which may misrepresent the taxonomic profile of microorganism communities. In this work, a methodology for the analysis and quantification of contaminants in low biomass samples was implemented. For this purpose, DNA was extracted from 34 tumoral renal tissue samples and 9 reagent-only samples, and amplified by nested PCR for a V3-V4 region of the 16S rRNA gene. A total of 5 PCR blank controls was also included. The 16S rRNA PCR products were sequenced in duplicate on an Illumina platform and sequencing reads were treated and processed using QIIME2. Taxonomy analysis revealed the presence of 19 phyla, 27 classes, 55 orders, 75 families, 89 genera and 114 distinct species in samples and controls. Using the Decontam R package, 35 contaminating genera were identified in the DNA extraction controls and 17 contaminating genera were found in the PCR controls. The proportion of contaminants in renal samples varied between 0.01% and 24,8%. Nine genera were not previously labeled as contaminants in the literature, indicating that the (re)identification of these microorganisms in other studies should be interpreted with caution. We conclude that contaminating strains are a serious problem in microbiome studies which use low biomass samples, and that the methodology presented here is an efficient approach to detect and quantify those strains.

2 - Hugo Barreto - PhD Student at Instituto Gulbenkian de Ciência

The Landscape of Adaptive Evolution of a Gut Commensal Bacteria in Aging Mice

Hugo C. Barreto 1, Ana Sousa 2 and Isabel Gordo 1

1 Instituto Gulbenkian de Ciências, Rua da Quinta Grande 6, 2780 Oeiras, Portugal, 2 iBiMed, Institute for Biomedicine, Universidade de Aveiro, Agra do Crasto, Edifício 30, 3810 Aveiro, Portugal



Aging is a complex process, with many associated time-dependent phenotypes. The gut microbiota have long been postulated as an important factor in shaping healthy aging. During aging, changes in the microbiota composition occur, with taxa that are rare in adults becoming dominant in the elderly. Increased inflammation associated with aging is also known to modulate and be modulated by the microbiota. Ecological interactions are known to affect the evolution of bacteria both in vitro and in vivo, but the extent to which these and the host age-dependent inflammatory environment can alter the pattern of evolutionary change of a gut commensal lineage is still unknown. Here, we provide the first genomic analysis of such evolution in cohorts of old mice, under controlled host genetics and lifestyle conditions. We find that Escherichia coli evolution when colonizing the gut of old mice significantly differs from its evolution in young mice. Evolution toward metabolic adaptation is slower in old than young mice, and mutational targets concerning stress-related functions were found specifically in the inflamed gut of old mice. Taking the genetic basis of E. coli short-term evolution as a reflection of the environment it experiences, the sequencing data indicate that aging imposes a more stressful environment to this important colonizer of the mammalian gut.

3 - João Rato - Bioinformatics Specialist at BioData.pt

The Microbiome Portal: A Platform to Unify Portuguese Microbiota Research

João Rato 1, Ricardo Leite 1, Daniel Faria 2,

1 Instituto Gulbenkian de Ciência, 2780 Oeiras, Portugal, 2 Instituto de Engenharia de Sistemas e Computadores - Investigação e Desenvolvimento (INESC-ID), 1000 Lisboa, Portugal

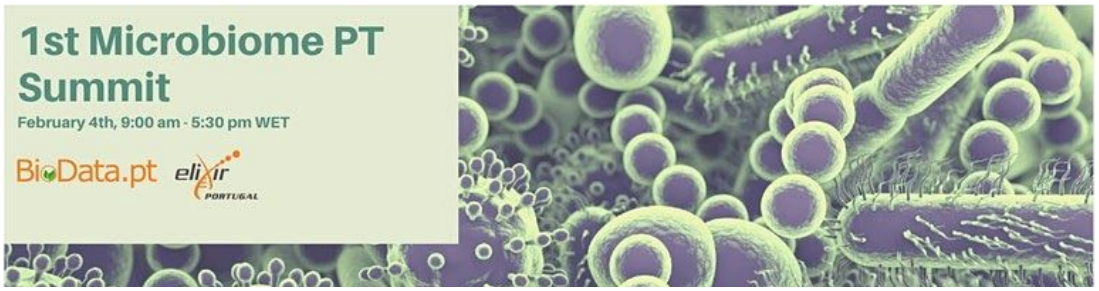
The Microbiome portal is an ongoing platform aiming to unify all the microbiota associated data generated by the Portuguese research community. The main goal of this project is to provide some accessory tools for metagenomic analysis while assisting researchers on data deposition and data shareability. Not all data is publicly accessible, users can define the kind of visibility for each project and share in different schemes (groups, projects) according to the needs. It strongly encouraged the metadata deposition, being some fields required while others are open for a better description of the data. The platform also provides analysis tools for the stored data where the user can choose different analysis pipelines, public and custom databases.

4 - Jorge de-Carvalho - Research Bioengineer at Instituto Gulbenkian de Ciência

Gut-on-chip - Microfluidics-based model for microbiome studies

Jorge de-Carvalho 1, 2

1 Gene Express Lda, 2645 Alcabideche, Portugal, 2 Instituto Gulbenkian de Ciência, 2780 Oeiras, Portugal



The gut enrols crucial body functions, such as metabolism, transport and absorption of nutrients and other molecules, but also plays an effective role on immune capability. The side-effects of several drugs on gut and the unknown causality behind cancer, inflammation or other diseases raise the need for effective screening models. Lastly, there is growing evidence for the influence of microbial symbionts populations on gut. Due to its biological complexity, we are far from getting a holistic comprehension of gut microenvironments and how they respond to different perturbations. Therefore, it is important to develop innovative reductionist methodologies that circumvent the limitations of conventional in vitro studies. The development of organ-on-chip microfluidic platforms will allow to decipher the underlaying principles of host-microbe interplay with precise control of culture conditions. Here, I will provide a flash overview about gut-on-chip devices potential for microbiome studies.

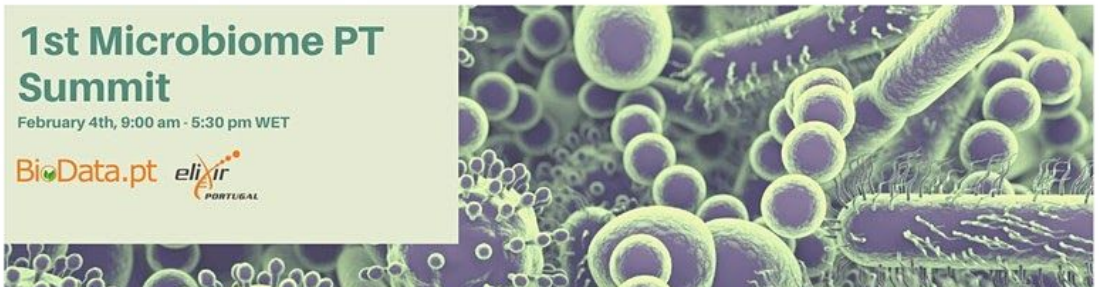
5 - Luísa Peixe - Associate Professor at Faculdade de Farmácia da Universidade do Porto

Urinary microbiome structure of healthy women: a comprehensive description supported in culturomics and amplicon sequencing

Svetlana Ugarcina-Perovic 1, Magdalena Ksiezarek 1, Joana Rocha 1, Márcia Sousa 1, Teresa Gonçalves Ribeiro 1, Filipa Grosso 1, Luísa Peixe 1

1 UCIBIO-REQUIMTE, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, 4050 Porto, Portugal

The recognition of a female urinary microbiota (FUM) engendered the need for comprehensive characterization of the urinary microbiota in health and disease. To improve our understanding of FUM, we analyzed midstream urine samples of twenty reproductive-age European healthy women using a combination of extended culturomis and long-reads third generation sequencing of the 16S rRNA gene V1-V8 regions. Community structure types (CST) were analysed by hierarchical clustering of Bray-Curtis dissimilarity distance matrices and compared by Mantel test. Alpha diversity was estimated by the Shannon index. The healthy FUM was characterized by wide bacterial species diversity (8 phyla, 115 genera and 290 species) shaping urinary CSTs. In median, we characterized 53 species/sample and unveiled 11 bacterial species not previously cultured from FUM (e.g., Gardnerella leopoldii, Gardnerella swidsinskii, Lactobacillus paragasseri), including putatively new Limosilactobacillus species. Only 64 species (22%) were detected by both methodologies. Alpha diversity depicted by culturomic approach varied from 0.002 to 2.66 (median H′ = 1.64) and by amplicon sequencing from 0.135 to 2.79 (median H′ = 0.90). The healthy FUM has a high degree of inter-individual variability at species level and we could not identify a single species common to all samples was identified. Nevertheless, 14 bacterial species were present in more than 50% of samples. The most prevalent CSTs were characterized by combinations of Lactobacillus spp. and Corynebacterium spp.,



followed by a CST with highly abundant Lactobacillus iners and single Gardnerella species (e.g., Gardnerella vaginalis). A moderate correlation ($r = 0.5$, $p < 0.05$) between the CSTs, at species level, assigned by culturomics and amplicon sequencing was observed, In this study we substantially enlarged urinary microbiome repertoire and highlight the complementarity of culturomic and amplicon sequencing approaches for comprehensive description of the urinary microbiome structure of healthy women.

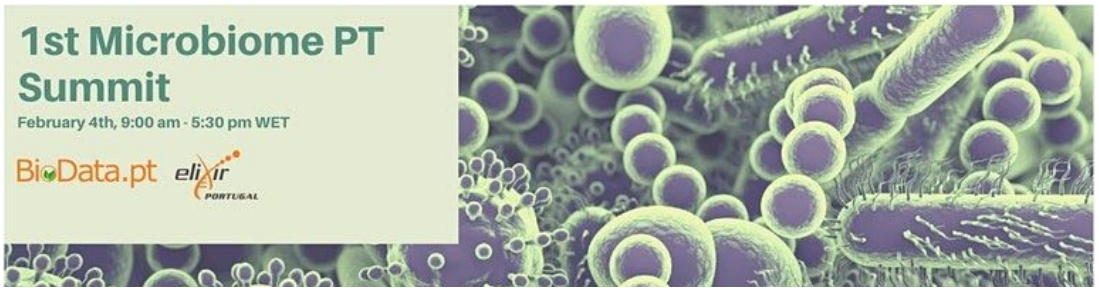
6 - Magdalena Ksiezarek - PhD Student at UCIBIO-REQUIMTE

Long-term dynamics of the urinary microbiota of healthy European women

Magdalena Ksiezarek 1, Svetlana Ugarcina-Perovic 1, Joana Rocha 1, Filipa Grosso 1, Luísa Peixe 1

1 UCIBIO-REQUIMTE, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, 4050 Porto, Portugal

Knowledge on healthy female urinary microbiota (FUM) has been, until now, mostly supported at genus level and at one time-point. However, profound species-level characterization of healthy FUM dynamics over time is essential for further correct interpretation of its role in healthy urinary tract. In this study, we investigated FUM shifts over a long period of time (within 2.5-year interval) in young European healthy women to understand species dynamics within healthy FUM.
Extended culturomics of voided midstream urine sample pairs with improved isolates' identification (MALDI-TOF MS and gene markers sequencing) revealed a mean Shannon diversity index of 1.25 and mean of 19 species/sample (range 5-39 species; total of 115 species; 1830 isolates). Overall, high species variability between individuals was captured by a variety of community structure types, with the largest cluster characterized by Lactobacillus crispatus, often in combination with Gardnerella vaginalis or Gardnerella genomospecies 3. Significant FUM composition differences, related to Finegoldia magna and Streptococcus anginosus, according to smoking status were found.
A high species variability within individuals (Shannon index SD > 0.5 in 7 out of 10 sample pairs) with a mean of 29% of shared species (range 9.1%-41.7%) was observed. Moreover, 4 out of 10 sample pairs clustered in the same community structure type. The stable FUM sample pairs presented high abundance of Lactobacillus crispatus, Streptococcus agalactiae or Lactobacillus paragasseri and Bifidobacterium spp.. Moreover, Gardnerella vaginalis, Gardnerella genomospecies 3 or Gardnerella swidsinskii were often maintained within individuals in high abundance.
Overall, long-term species dynamics was frequently observed among urinary microbiota of European young healthy women. This suggests possible interchange of particular species in healthy FUM and the existence of multiple health-associated FUM compositions in certain individuals. Additionally, we also provided evidence on



resilience of particular bacterial communities and exposed species more prone to persist in lower urinary tract.

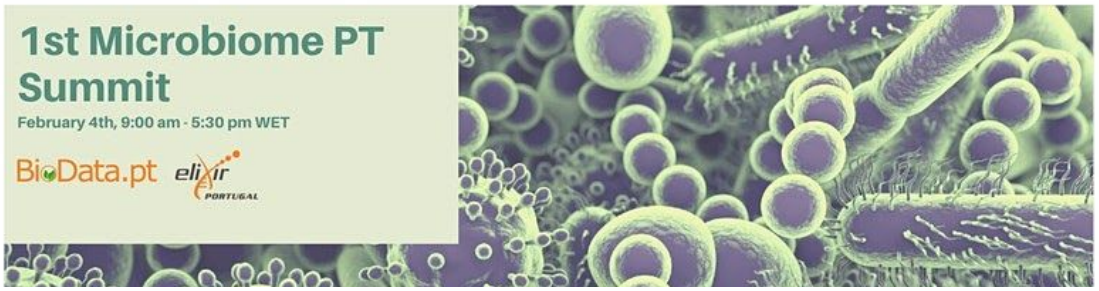
7 - Márcia Sousa - PhD Student at UCIBIO-REQUIMTE

Gardnerella species in the healthy female urinary microbiome – a step forward in understanding their diversity and interactions

Márcia Sousa 1, Magdalena Ksiezarek 1, Filipa Grosso 1, Teresa Ribeiro 1, Luísa Peixe 1

1 UCIBIO-REQUIMTE, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, 4050 Porto, Portugal

Recent advances in DNA sequencing and culture-based methodologies allowed the identification of a female urinary microbiome (FUM). However, the role of the genus Gardnerella remains controversial since it was described in the genitourinary microbiome of women with/without urinary symptoms. Moreover, the identification of Gardnerella species in FUM is still unclear as its taxonomy was recently reviewed, with four species (Gardnerella vaginalis, Gardnerella leopoldii, Gardnerella piotii and Gardnerella swidsinskii) and 9 genomospecies being proposed. We aimed to elucidate the occurrence of Gardnerella species in the healthy urinary microbiome of reproductive-age women. Twenty midstream urine samples (0.1 mL) cultured in Columbia with 5% sheep blood and chromogenic media were incubated (37°C-48h; aerobic, microaerophilic and anaerobic conditions). Isolates were initially identified by VITEK® MS and Gardnerella was confirmed by PCR and sequencing of cpn60. Relative abundance (RA) was calculated based on colony forming units/ml. cpn60-phylogenetic tree was constructed using MEGA software. Gardnerella was identified in 30% (n=6/20) of the samples, RA ranging from 1-92%. Four clusters were identified by cpn60-phylogenetic tree (bootstrap values >93%): i) G. piotii/genomospecies 3 (n=2 samples; RA=60% or 1%), ii) G. swidsinski (n=2; 49% or 32%), iii) G. vaginalis (n=2; 92% or 4%), and iv) G. leopoldii (n=1; 24.5%). Co-occurrence of different Gardnerella species (G. swidsinskii and G. piotii/genomospecies 3) and other high abundant bacteria were also observed (G. leopoldii, Alloscardovia omnicolens, and Bifidobacterium spp.; G. swidsinskii and Lactobacillus mulieris/Atopobium vaginae, G. piotii/genomospecies 3 and Atopobium vaginae). Our results suggest an important role of different Gardnerella species in healthy FUM. Moreover, we unveil particular bacterial combinations comprising Gardnerella spp. which interactions might contribute for a healthy urinary tract in reproductive women. Finally, from our phylogenetic analysis, G. piotii and genomospecies 3 may correspond to the same species.



8 - Rafael Santos - Project Manager at BioData.pt

Biome-Shiny: A Shiny R app for microbiome visualization

Henrique Costa 1, Rafael Santos 1, Ricardo Leite 1

1 Instituto Gulbenkian de Ciência, 2780 Oeiras, Portugal

Biome-Shiny is a microbiome analysis pipeline, primarily based around the "phyloseq" and "microbiome" R libraries, as they provide a fairly extensive package for microbiota analysis. This pipeline was built within a user-friendly graphical interface, developed with the R “shiny” library, to generate and visualize community composition and diversity through interactive plots allowing wet lab researchers to easily perform their analyses with significant output recalculation and reproducibility. It takes as an input a .biom file and a .csv file that may include sample metadata. It allows users to filter out the dataset, removing non-relevant samples and taxa from the data, as well as allowing users to rarefy the dataset, removing potential bias caused by skewed abundance distributions.
To visualize the most abundant OTUs/ASVs/species, the application generates a heatmap of species abundance per sample. For a more complete visualization of microbial composition per sample, it offers a barplot with (relative and absolute) abundance of all species present in the samples. It also includes visualization options for both alpha and beta diversity, as well as a PERMANOVA statistical analysis of the dataset.
Biome-Shiny is currently distributed as an individual application, as an R package and as a Docker image for ease of deployment.

Microbiome communities in the sea and other aquatic environments

9 - Ana Coelho Alão Freitas - PhD Student at ITQB-UNL

Metagenomic Analysis in Extreme Environments for the Discovery of Novel Enzymes and Pathways

Ana Alão Freitas 1, Ricardo Leite 2, José B. Leal 3, Isabel Rocha 1

1 Instituto de Tecnologia Química e Biológica António Xavier da Universidade Nova de Lisboa, 2780 Oeiras, Portugal 2 Instituto Gulbenkian de Ciência, 2780 Oeiras, Portugal, 3 Centro de Estudos de Doenças Crónicas (CEDOC) da Faculdade de Ciências Médicas da Universidade Nova de Lisboa, 1150 Lisboa, Portugal

Abstract: A large number of industrial processes occur in environments with high temperatures, extreme pH values and in the presence of inhibitor compounds, which makes the use of enzymes obtained from moderate media unfeasible in these processes. Thus, the use of enzymes selected from extreme environments can

facilitate and increase the number of enzymes applied in a large number of industrial processes and improve some of the already existing ones. These environments are composed by microbial communities difficult to study, due to the specific habitats and because only few species can be isolated and cultivated with the existing techniques. For these reasons, over the last few years metagenomics has been widely used to characterize the taxonomic and genetic diversity of these communities. The use of metagenomics can allow the identification of enzymes that exhibit activity under extreme conditions that are similar to the ones occurring in industrial processes, and those enzymes can later be easily synthesized in different hosts. Given the high abundance of these extreme environments due to volcanic origin and the presence of secondary volcanic manifestations such as hydrothermal vents, the Azorean region is highly promising for developing these studies. In this work, we present a metagenomics pipeline to perform a wide characterization of the genetic diversity of shallow hydrothermal vents from Azores. That can be later used to foster identification of novel enzymes and pathways with industrial applications.

Keywords: microbial communities ; shallow hydrothermal vents; Azores

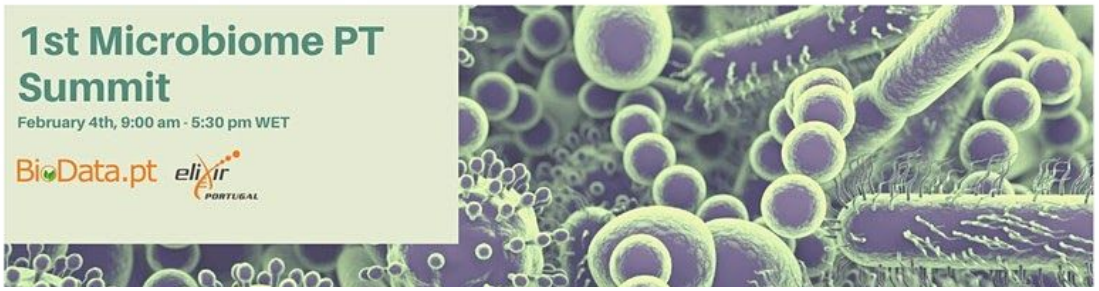
10 - Ana Pereira - PhD Candidate at CIBIO-InBIO

To clean or not to clean: cleaning activity predicts cleaner fish skin microbiota diversity and pathogen abundance

Ana Pereira 1, Marta Cardoso Soares 1, Teresa Santos 1, Ana Poças 1, Pauline Narvaez 1, Amy Apprill 1, Paul Sikkel 1, Raquel Xavier 1

1 CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485, Vairão, Portugal

Microbial transfer between interacting partners is common with physical proximity and known to shape skin and gut microbial consortia. Although beneficial outcomes of microbial transmission within social groups have been described, such as increased disease resistance or increased digestive capacity, social interactions may also facilitate pathogen transmission. Importantly, social microbial transmission should increase microbiota complexity, promoting competition and regulating abundance of opportunistic and/or pathogenic taxa. Despite the increased popularity of fish as models to study social cognition and behaviour, the effects of sociality on fish microbiota have not yet been studied. Cleaning mutualisms are one of most iconic and well-studied fish interactions. Some of the best-known examples of cleanerfish are the Caribbean cleaning gobies, which are usually found at cleaning stations and are visited by several different client species per day to be “inspected” by cleaners. Regardless of the obvious benefits for clients (ectoparasite removal), the gain of an easy meal for cleaners may come with a cost, such as bacterial contamination from potentially diseased clients. Here we examined the relation between cleaning activity and microbiome composition and structure of the most ubiquitous Caribbean cleaning goby,



Elacatinus evelynae, in four reefs in the US Virgin Islands using a metataxonomic approach. Overall, cleaners' microbial alpha diversity increased with clients' traffic, as well as with the number of clients inspected. Moreover, client diversity (i.e., number of client genera) also impacted alpha diversity with the same trend. Although microbial structure varied amongst cleaners from different locations, the most abundant taxa were dominated by Psychrobacter and Pseudomonas, which are both known to be potential fish pathogens. Those and other potential pathogenic bacteria were more prevalent in locations with higher cleaning activity, suggesting a possible cost for cleaners with higher cleaning activity, which could be to some degree counterbalanced by higher alpha diversity.

11 - Daniela Rosado - PhD Student at CIBIO - InBIO

Longitudinal sampling of external mucosae in farmed European seabass reveals complex bacterial dynamics and water temperature effects

Daniela Rosado 1, Raquel Xavier 1, Jo Cable 2, Ricardo Severino 3, Marcos Pérez-Losada 1,4

1 CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485, Vairão, Portugal, 2 - School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK, 3 Piscicultura Vale da Lama, Sapal do Vale da Lama, Odiáxere, 8600, Lagos, Portugal, 4 Computational Biology Institute, Department of Biostatistics and Bioinformatics, Milken Institute School of Public Health, George Washington University, Washington DC, 20052, USA

Microbiota is intrinsically linked to fish health and fitness, being influenced by biotic and abiotic factors. Fish microbiotas, particularly those of external mucosa (skin and gills), have been described as being highly variable. Water temperature in particular can influence the microbiota of external mucosae of fish, such as the skin and gills, namely by limiting bacterial adhesion and growth. Thus temperature has been correlated with fish microbial diversity and bacterial infections. Aquaculture is heavily affected by infectious diseases, especially in warmer months when outbreaks mostly occur. Importantly, aquaculture practices are known to promote stress and microbial dysbiosis, leading to an increased abundance of potentially pathogenic bacteria. In this regard, fish mucosa health is extremely important as it acts as a primary barrier against pathogens. We used 16 rRNA V4 metataxonomics to characterize the skin and gill microbiotas of the European seabass, Dicentrarchus labrax, and the surrounding water over 12 months, assessing the impact of the water temperature on microbial diversity and function. The results showed that the microbiotas of external mucosae were highly dynamic with similar longitudinal trends in diversity. Several potentially pathogenic genera were highly abundant, showing complex interactions with other genera, some of which with recognized probiotic activity. The surrounding water temperature exerted an influence in the diversity and function of the studied tissues, both at short and wider

time frames. Additionally, dysbiosis was more frequent in warmer months and in transitions between cold and warm months. The results show the need for longitudinal studies to evaluate the impacts of environmental factors on the microbiota of farmed fish.

12 - Francisco Pascoal - PhD Student at CIIMAR

Advances in the description of the prokaryotic rare biosphere in the Arctic Ocean

Francisco Pascoal 1, Rodrigo Costa 2, Catarina Magalhães 1

1 Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, 4450 Matosinhos, Portugal, 2 Instituto de Bioengenharia e Biociências (iBB), Instituto Superior Técnico (IST), Universidade de Lisboa, 1049 Lisboa, Portugal

The prokaryotic rare biosphere represents the low abundance populations of prokaryotes and is important for ecosystem resistance and resilience. The few previous studies on the prokaryotic rare biosphere of the Arctic Ocean assert that water masses harbor specific microbial ecotypes and most prokaryotic taxa remain rare across biogeography and seasons. For the purpose of uncovering the different types of rare populations in the Arctic Ocean and respective dynamics during a winter to spring transition, we used seawater samples from the Norwegian Young Sea Ice Expedition (2015). Our results found that most of the rare taxa were transiently rare, meaning that they appear and disappear in different samples, probably as a result of dispersal limitation caused by the different water masses. Furthermore, we suggest that conditional rarity, which represents taxa that vary between abundant and rare across time/space, are more prevalent at epipelagic layers, where seasonal variation in light availability occurs. Rare prokaryotes tend to maintain low abundance within specific Arctic Ocean water masses, but they can become abundant or disappear across seasonal variation and different water masses. Future work should tackle how the rare biosphere responds to climate change and which are the consequences of this response for ecosystem functioning.

13 - Gabriela Simões - Research Fellow at CNC-UC

Analysis of the functional metagenome of two Portuguese hot springs identifies the potential for compatible solutes synthesis

Gabriela Simões 1, Nadine Castelhano 1, Cristina Barroso 1, 2, Luciana Albuquerque 1, Conceição Egas 1, 2

1 Center for Neuroscience and Cell Biology, University of Coimbra, 3000 Coimbra, Portugal 2 Next Generation Sequencing Unit, Biocant, 3060 Cantanhede, Portugal

Hot spring waters are important ecosystems inhabited by unique bacteria and archaea. Survival of microorganisms in these environments implies their ability to adapt to

adverse conditions such as chemical properties and high temperature. Accumulation of compatible solutes is one of the adaption mechanisms thermophiles use in these environments, and some of these compounds have biotechnological applications. Here we studied the metagenomes of Portuguese hot springs waters from Chaves and S. Pedro do Sul, focusing on the potential for compatible solutes synthesis. We identified enzymes involved in the synthesis of glucosylglycerate and mannosylglucosylglycerate only in Chaves, and trehalose in the two springs. The enzymes were detected in microorganisms where the accumulation of compatible solutes has not been described yet. The trehalose bifunctional enzyme was detected in bins of the Aquificae phylum. The enzymes for the synthesis of glucosylglycerate were identified in bins from Acidobacteria, Nitrospirae and Deltaproteobacteria. A mannosylglucosylglycerate synthase and a glucosyl-3-phosphoglycerate synthase were detected in a bin from the Chloroflexi phylum. The enzymes were similar to those of the Caldilineaceae and Anaerolineaceae families and may suggest a new pathway for the synthesis of mannosylglucosylglycerate. Identifying genes for the synthesis of compatible solutes in the sequence space in microorganisms not yet known to have specific enzymatic activities represents a powerful tool for in silico screening and selection of gene candidates for functional testing. Additional studies are required to characterize the enzymes identified.

Funded by projects H2020 Metafluidics - 685474, FCT - UIDB/04539/2020 and GenomePT- POCI-01-0145-FEDER-022184.

<u>14 - Gracinda Sanches-Fernandes - PhD Candidate at iBB</u>

Effects of live feed manipulation with antimicrobial metabolites on fish larvae microbiome assembly: a molecular-based assessment

Gracinda M.M. Sanches-Fernandes 1, Gianmaria Califano 2, 3, Sara Castanho 4, Florbela Soare 4, Laura Ribeiro 4, Leonardo Mata 2 and Rodrigo Costa 1, 2, 5

1 Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico (IST), Universidade de Lisboa, 1049 Lisbon, Portugal, 2 Centre of Marine Sciences, University of Algarve, 8005 Faro, Portugal, 3 Institute for Inorganic and Analytical Chemistry, Friedrich-Schiller-Universität Jena, Jena, Germany, Portuguese Institute for the Ocean and Atmosphere, Aquaculture Research Station, 8700 Olhão, Portugal, Lawrence Berkeley National Laboratory, Department of Energy-Joint Genome Institute, (DOE-JGI), UC Berkeley, USA

Opportunistic bacteria acquired through rearing water or live feed ingestion are believed to underpin high mortality rates of fish larvae, constituting a production bottleneck for the aquaculture industry. We employed 16S rRNA gene sequencing to determine whether treatment of live feed (rotifers and artemia) with algal-derived,

anti-bacterial metabolites could alter bacterial community structure of gilthead seabream (Sparus aurata) larvae in a larviculture facility. Owing to a large degree of sample-to-sample variation, pronounced “legacy effects” of live feed manipulation on the total fish larvae bacteriome could not be verified. Notwithstanding, some phylotypes representing opportunistic taxa such as Stenotrophomonas, Pseudomonas and Klebsiella displayed reduced abundances in the bacteriome of fish larvae fed metabolite-treated vs. control live feed. Some potentially beneficial phylotypes in the Alphaproteobacteria clade (e.g., Paracoccus sp., Polymorphum gilvum, Rhodobacteraceae sp.) were consistently – although not significantly – promoted in the treated larval samples. Our approach induced shifts in relative abundance of specific bacterial phylotypes in the fish host, encouraging future microbiome manipulation attempts to improve fish larviculture. Successful host colonization and competition with resident symbionts are primary barriers that need to be overcome if live feeds are to be used as efficient probiotic delivery systems to fish larvae in the future.

Keywords: aquaculture, bacterial diversity, fish microbiome, host-microbe interactions, microbial therapy.

15 - Mafalda Baptista - Researcher at CIIMAR

Estuarine microbiome response to copper oxide nanoparticles

Mafalda S. Baptista 1, 2, 3, António G. G. Sousa 1, Aldo Barreiro 1, Catarina Magalhães 1, 2, 4, 5

1 CIIIMAR/CIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal, 2 Faculdade de Ciências, Universidade do Porto, Porto, Portugal, 3 International Centre for Terrestrial Antarctic Research, University of Waikato, Hamilton, New Zealand, 4 School of Science, University of Waikato, Hamilton, New Zealand, 5 Ocean Frontier Institute, Dalhousie University, Halifax, Nova Scotia, Canada

Nanomaterials, particularly metallic nanoparticles (NPs), are increasing in usage in multiple fields. Methods for their removal from wastewaters are yet developing and currently a significant fraction of escapes treatment plants. As a result estuaries become the final repository for metal NPs. The implications towards estuarine biota have been assessed, but usually at concentrations much higher than the environmentally-predicted to occur. Moreover, while toxicity has often been assessed, the impact of metal NPs towards ecosystem services has seldom been reported. The aim of this study was to ascertain how the deposition of copper oxide nanoparticles (Cu NPs) in estuarine sediments will change metal speciation and bioavailability, which in turn will impact the functionality of estuarine archaeal and bacterial communities. For this we selected the Douro River estuary in Portugal (North Atlantic), and performed a series of microcosms enrichment experiments exposing estuarine sediments to Cu

NPs of size <50 nm, at a concentration of 10 ng/g, for 4 h, 7h and 24 h. The microcosms pH and dissolved oxygen was monitored, as well as concentrations of nitrate, nitrite and ammonium. DNA was extracted from the sediment and sequencing was performed using Illumina Hiseq Xten to acquire 150 bp paired-end sequences. This data was used to identify links between the microbial community and ecosystems services they might provide, namely the service of removing fixed nitrogen which is critical in eutrophic ecosystems.

16 - Miguel Semedo - Postdoctoral Researcher at CIIMAR

Depth profile of nitrifying prokaryotes in the oligotrophic North Pacific

Miguel Semedo 1, Eva Lopes 1, Mafalda S. Baptista 1, 2, 3, Ainhoa Oller 4, Javier Gilabert 4, Maria Paola Tomasino 1, Catarina Magalhães 1, 2, 5

1 Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, 4450 Matosinhos, Portugal 2 Faculty of Sciences, University of Porto, 4000 Porto, Portugal, 3 International Centre for Terrestrial Antarctic Research, University of Waikato, 3240 Hamilton, New Zealand, 4 Department of Chemical & Environmental Engineering, Universidad Politécnica de Cartagena (UPCT), 30201, Cartagena, Spain, 5 School of Science, University of Waikato, 3240 Hamilton, New Zealand

Nitrification is a vital ecosystem function in the open ocean that promotes nitrogen regeneration and primary production. The distribution of nitrifying organisms in the remote oligotrophic ocean and their interactions with the physicochemical environment are relatively understudied. In this work, we aimed to evaluate the depth profile of nitrifying archaea and bacteria in the Eastern North Pacific Subtropical Front, an area with limited biological surveys but with intense trophic transferences and physicochemical gradients. Furthermore, we investigated the dominant physicochemical and biological relationships within and between the two groups of nitrifying organisms, ammonia-oxidizing archaea (AOA) and nitrifying bacteria (AOB and NOB). We used 16S rRNA gene sequencing to identify and characterize the nitrifying groups within the first 500 m of the water column and to analyze their physicochemical and biological interactions. The water column was characterized by two contrasting environments, warm O2-rich surface waters with low dissolved inorganic nitrogen (DIN) and a cold O2-deficient mesopelagic layer with high concentrations of nitrate (NO3-). Thaumarcheotal AOA and bacterial NOB were highly abundant below the deep chlorophyll maximum (DCM) and in the mesopelagic. In the mesopelagic, AOA and NOB represented up to 25% and 3% of the total prokaryotic community, respectively. The AOA community in the mesopelagic was dominated by unclassified genera that may constitute a novel group of AOA highly adapted to the conditions observed at those depths. Additionally, a large network of positive interactions was found between putative nitrifying ASVs, including 1845 significant correlations and 19 sub-communities of AOA and NOB, irrespective of their taxonomic

classification. This study provides new insights into our understanding of the roles that AOA may play in recycling inorganic nitrogen in the oligotrophic ocean, with potential consequences to primary production in these remote ecosystems.

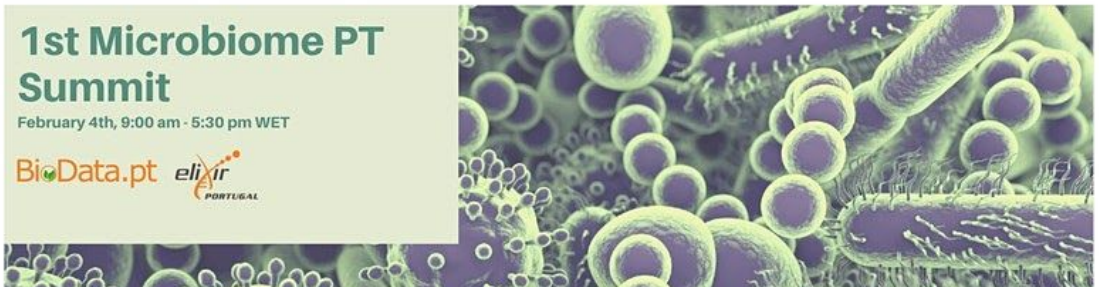
17 - Nuno Borges - Researcher at iBB

Identification and characterization of bacteria with hydrolytic and antibacterial activities isolated from gilthead sea bream (Sparus aurata) for application as probiotics in larviculture

Nuno Borges 1, André Magalhães 1, Florbela Soares 2, and Rodrigo Costa 1, 3, 4, 5

1 Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, 1049 Lisbon, Portugal, 2 Portuguese Institute for the Ocean and Atmosphere, Aquaculture Research Station, Olhão, Portugal, 3 Centre of Marine Sciences, Algarve University, 8005 Faro, Portugal, 4 Department of Energy, Joint Genome Institute, Berkeley, California 94720, USA, 5 Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

Increasing fish disease resistance and promoting fish growth are crucial parameters to improve economic sustainability of aquaculture. Bacterial infections are more prevalent during fish larviculture since the host's immune system is underdeveloped. Microbiome manipulation during early fish developmental stages through probiotic supplementation is a promising strategy to control pathogens in aquaculture. The aim of this study was to isolate bacteria from Sparus aurata and determine their hydrolytic enzyme biosynthesis capacities and antagonistic activities against fish pathogens in vitro for the selection of promising probiotic candidates. Bacterial cultivation was carried out using different culture media (diluted R2A, TSA, and MRS) and fish developmental stages (eggs, larvae, and juvenile guts). A total of 102 strains were isolated and identified. From the fertilized eggs through larval to the juvenile stage, the microbiome was composed of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria species. While Proteobacteria was the dominant phylum across all developmental stages, Firmicutes increased and Bacteroidetes decreased in abundance in the juvenile stage. Based on literature reports, 35 strains were classified as non-pathogenic and characterized for several probiotic activities. Twenty-six strains exhibited at least one hydrolytic extracellular enzyme activity (i.e., lipases, proteases, amylases, and cellulases) and 8 strains were able to produce all four hydrolytic enzymes. These features are especially important for the digestion of food ingredients and for their growth on the fish gut. A few isolates showed promising antimicrobial activities against Photobacterium damselae and Streptococcus iniae. However, no growth inhibition of Vibrio parahaemolyticus was observed. Metabolite extracts from the cultures will be further tested to obtain further insights into the antimicrobial mechanisms. Isolates displaying prominent hydrolytic enzyme and antimicrobial activities will be tested as



candidate probiotics for the control of pathogenic bacteria and promotion of fish growth in larviculture experiments.

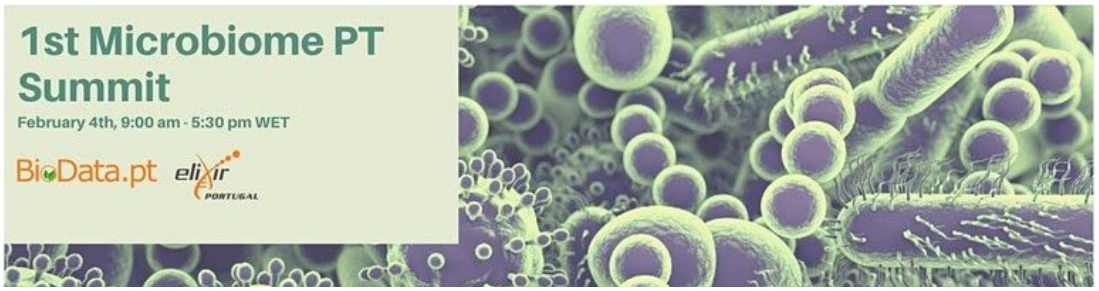
18 - Rúben Silva - PhD Student at iBB

Functional metagenomics reveals differential chitin degradation and utilization features across free-living and host-associated marine microbiomes

Silva, R. 1, Raimundo, I. 1, Meunier, L. 1, 2, Valente, S.M. 1, Lago-Lestón, A. 3, Keller-Costa, T. 1 and Costa, R. 1, 4, 5

1 Instituto de Bioengenharia e Biociências, Instituto Superior Técnico (IST), Universidade de Lisboa, 1049 Lisbon, Portugal,2 Laboratory of Aquatic Systems Ecology, Université Libre de Bruxelles, Brussels, Belgium, 3 Department of Medical Innovation, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), 22860, Ensenada, Mexico, 4 Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005 Faro, Portugal, 5 Department of Energy, Joint Genome Institute, Walnut Creek, California 94598, USA, and Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA.

Chitin ranks as the most abundant polysaccharide in the oceans yet knowledge of shifts in structure and diversity of chitin-degrading communities across marine niches is scarce. Here, we integrate cultivation-dependent and -independent approaches to shed light on the chitin processing potential within the microbiomes of marine sponges, octocorals, sediments and seawater. We found that cultivatable host-associated bacteria in the genera Aquimarina, Enterovibrio, Microbulbifer, Pseudoalteromonas, Shewanella and Vibrio were able to degrade colloidal chitin in vitro. Congruent with enzymatic activity bioassays, genome-wide inspection of cultivated symbionts revealed that Vibrio and Aquimarina species, particularly, possess several endo- and exo-chitinase-encoding genes underlying their ability to cleave the large chitin polymer into oligomers and dimers. Conversely, Alphaproteobacteria species were found to specialize in the utilization of the chitin monomer N-acetylglucosamine more often. Phylogenetic assessments uncovered a high degree of within-genome diversification of multiple, full-length endo-chitinase genes for Aquimarina and Vibrio strains. We then analysed the abundance distributions of chitin metabolism-related genes across 30 Illumina-sequenced microbial metagenomes and found that the endosymbiotic consortium of Spongia officinalis is enriched in polysaccharide deacetylases, suggesting ability of the marine sponge microbiome to convert chitin into its deacetylated form chitosan. Instead, the abundance of endo-chitinase and chitin-binding protein encoding genes in healthy octocorals levelled up with those from the surrounding environment but was found to be depleted in necrotic octocoral tissue. Using cultivation-independent, taxonomic assignments of endo-chitinase encoding genes, we unveiled unsuspected richness and divergent structures of chitinolytic



communities across host-associated and free-living biotopes, revealing putative roles for uncultivated Gammaproteobacteria and Chloroflexi symbionts in chitin processing within marine invertebrates. Our findings suggest that differential chitin degradation pathways, utilization and turnover dictate the processing of chitin across marine micro-niches and support the hypothesis that inter-species cross-feeding could facilitate the co-existence of chitin utilizers within marine invertebrate microbiomes.

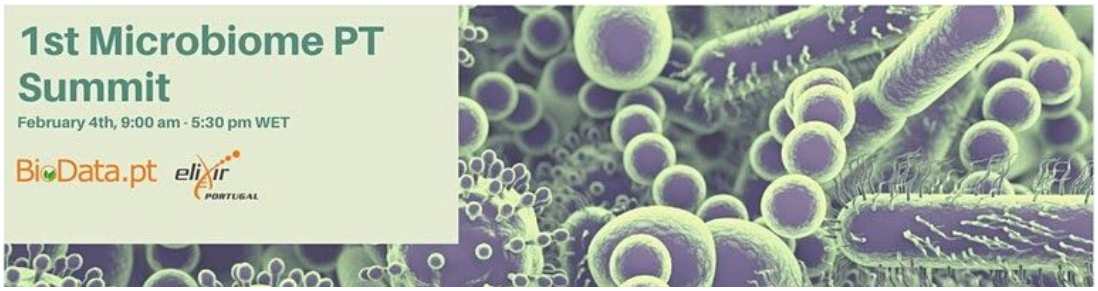
19 - Sandra Godinho Silva - PhD Student at iBB

Hidden treasures - High throughput mining of secondary metabolite biosynthetic gene clusters in the Flavobacteriaceae family

Sandra Godinho Silva 1, Masun Nabhan Homsi 2, Tina Keller-Costa 1, Ulisses Nunes da Rocha 2 and Rodrigo Costa 1

1 Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico da Universidade de Lisboa, 1049 Lisbon, Portugal, 2 Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Flavobacteriaceae spp. are historically well known for their capacity to degrade a wide variety of high molecular weight compounds which, in marine environments, make them key players of the marine carbon cycle. These bacteria have been usually regarded to possess a poor secondary metabolism, a view that may have been biased by our impaired ability to locate the genetic determinants of natural product biosynthesis. In this study, we compare all Flavobacteriaceae spp. genomes publicly available to date, and their respective metadata, to gain a broader perspective on the family's core genome functions as well as on differentiating secondary metabolite features that can give important insights into their ecological functions. Through the use of the most recent computational biology and dedicated genome mining tools, such as antiSMASH and BiG-SCAPE, we shed light on two particular Flavobacteriaceae genera, namely Aquimarina and Kordia, that possess an untypically high number of secondary metabolite biosynthetic gene clusters. These marine genera also possess larger genomes sizes and an increased repertoire of peptidases that may be a key factor enabling them to thrive in oligotrophic environments, like open seawater, where carbon availability is reduced. Noticeably, both Kordia and Aquimarina species have already been described as pathogens of algae and crustaceans, respectively. We hypothesize that versatile peptidase capabilities, summed with the ability to synthesize a wide variety of secondary metabolites with potentially different biological activities, may be directly related to an opportunistic lifestyle, enabling Aquimarina and Kordia species to cope with a wide range of constraints imposed by their immediate physico-chemical environment, and therefore, to thrive in multiple, and rather distinct, microniches.



20 - Tina Keller-Costa - Research Scientist at iBB

Functional and Taxonomic Signatures of the Microbiomes in Healthy versus Diseased Octocoral Tissue

Keller-Costa, T. 1, Lago-Lestón, A 2, Saraiva, J.P. 3, Toscan, R. 3, Silva, S.G. 1, Gonçalves, J. 4, Cox, C.J. 4, Kyrpides, N. 5, Nunes da Rocha, U. 3 and Costa, R. 1,4,5

1 Instituto de Bioengenharia e Biociências (iBB), Instituto Superior Técnico (IST), Universidade de Lisboa, 1049 Lisboa, Portugal, 2 Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), 22860, Ensenada, Mexico, 3 Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany, 4 Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005 Faro, Portugal, 5 Department of Energy, Joint Genome Institute, Walnut Creek, California 94598, USA, and Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

In octocorals (Cnidaria, Octocorallia), the functional relationship between host health and its symbiotic consortium is yet to be unveiled. Here, we employed comparative metagenomics to uncover the distinct functional and phylogenetic features of the microbiomes of healthy and necrotic Eunicella gazella tissues, healthy Eunicella verrucosa and Leptogorgia sarmentosa tissues, surrounding seawater and sediments.
Multivariate analyses based on 16S rRNA genes, Clusters of Orthologous Groups of proteins (COGs) and Protein families (Pfams) annotated from 20 Illumina-sequenced metagenomes each revealed separate clustering of the prokaryotic communities of healthy tissue samples of the three octocoral species from those of necrotic E. gazella tissue and surrounding environments. While the healthy octocoral microbiome was distinguished by so-far uncultivated Endozoicomonadaceae, Oceanospirillales and Alteromonadales phylotypes in all host species, a pronounced increase of Flavobacteriaceae and Alphaproteobacteria, originating from seawater, was observed in necrotic E. gazella tissue. Increased abundances of eukaryotic-like proteins, restriction endonucleases, CRISPR/Cas proteins, and genes encoding for heat-shock proteins, ion transport and iron storage, distinguished the prokaryotic communities of healthy octocoral tissue. An augmentation of arginase and nitric oxide reductase genes, observed in necrotic E. gazella tissues, suggests the existence of a mechanism for suppression of nitrite oxide production by which octocoral pathogens may overcome the host's immune system.
This is the first study to employ primer-less, shotgun metagenome sequencing to unveil the functional features of prokaryotic communities in octocorals. Our analyses reveal that the octocoral microbiome is sharply distinct from environmental surroundings, is host genus-specific and undergoes complex structural changes in the transition to the dysbiotic state. Host-symbiont recognition, abiotic-stress response, micronutrient acquisition and sophisticated antiviral defence mechanisms are signatures of prokaryotic communities in octocorals. These features may contribute to the

stabilization of symbiosis in the octocoral holobiont and constitute beneficial traits which can guide future studies on coral reef conservation and microbiome therapy.

Microbiomes of Plants

21 - Helga Monte - Master student at UNIRIO (Universidade Federal do Estado do Rio de Janeiro)

Metagenomics in microbial bioremediation of pollutants: gaps diagnosis

Helga Assumpção Birkner do Monte 1 & Natascha Krepsky 1

1 Neotropical Biodiversity Postgraduate Program, UNIRIO, Brazil

Bioremediation is a friendly technique for the degradation of pollutants in different environments. By using one or more species of microorganisms, molecular tools have proven beneficial in this area of knowledge. The connection between the NGS (next sequencing generation) methodology and metagenomics has popularized studies involving genetic material analyses of microorganisms present in an environmental sample, the identification of species that assemble this microbiome, and the cataloguing of microorganisms in reference databases in studies of pollutant degradation. The aim of this bibliographic survey is to diagnose possible gaps in the knowledge of metagenomics for microbial bioremediation of pollutants. The bibliographic survey was carried out through the PUBMED, using the keywords "bioremediation" and "microorganism" and "metagenomic" and "pollutant". From a total of 292 articles published in indexed journals, eight were selected for this preliminary analysis. From 2010 to 2020, the main limitations cited in studies were related to the ability to identify unknown or wrongly identified local bacteria, underestimating the diversity of microorganisms present in the analyzed sample. Another challenge was the inability of metagenomics to reveal the interactions between the microbiome and the environment. Thus, the integration of different omics (metagenomic, metatranscriptomic, metabolomic and metaproteomic) is necessary for a systemic study that allows the analysis of the regulation of the genetic level for bioremediation.

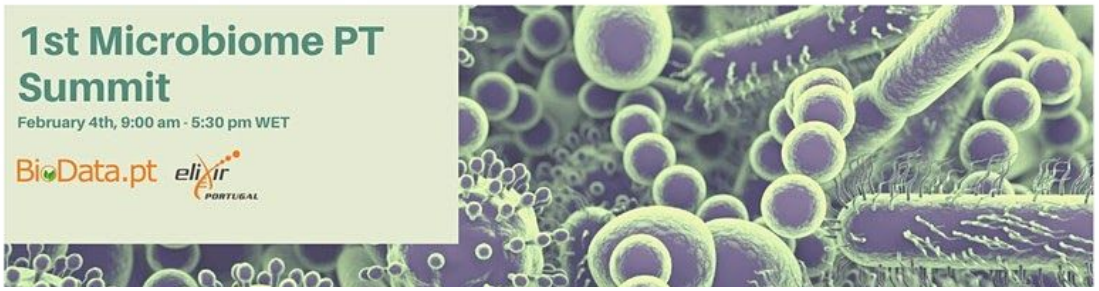
Keywords: next sequencing generation, metagenomics, microbial bioremediation, pollutants

22 - Isabel Natalia Serra Garcia - Research Associate at Universidade de Aveiro

Plant microbiota of coastal halophyte Salicornia ramossisima

Isabel N. Sierra-Garcia 1, Maria J. Ferreira 1, Sandro Figueiredo 1, Newton Gomes 1, Carla Patinha 2, Helena Silva 1 and Angela Cunha 1

1 Department of Biology & Center for Environmental and Marine Studies (CESAM), University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal, 2



Geosciences Department, Geobiotec, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

Plant associated microbial communities are considered crucial in the adaptation of halophytes to coastal environments. The plant microbiota can be horizontally acquired from the environment, or vertically transmitted from generation to generation via seed. Recruiting of the microbial communities by the plant is affected by geographical location, soil source, host genotype, and cultivation practice. There is limited knowledge reported on microbial community in halophytes and the influence of environment factors. In this work, the microbiota associated with the halophyte Salicornia ramossisima was investigated in two contrasting sites where S. ramossisima is established in the estuarine system of the Ria de Aveiro. One site corresponds to a natural salt marsh where S. ramossisima and other halophytes are present in wild and milder salinity conditions, and the other site is a former salt pan with higher soil salinity that nowadays is subjected to intensive crop production of S. ramossisima. Bacterial communities from rhizosphere, seeds and root endosphere of S. ramossisima from both sites was investigated by sequencing bacterial 16S rRNA gene using Illumina MiSeq platform. The analysis of the sequences showed that the three plant-associated compartments, rhizosphere, root endosphere, and seed endosphere harbor a distinct microbiome. Bacterial richness and diversity was higher in rhizosphere in both sites, followed by seed endophytes in the natural site, while seeds of crop site accounted the lowest values. Betadiversity measures indicated that bacterial communities in seeds differed by local while endosphere and rhizosphere were more similar between each other. Bacterial members of the classes Alphaprotebacteria and Bacteroidia were the most ubiquitous across sites and compartments and might encompass members of the core microbiome. This study provides a better understanding into the composition of the plant microbiota of S. ramossisima from saline environments in naturally occurring plants that might help them to gain tolerance to harsh environments.

23 - Nadine Castelhano - PhD Student at CNC-UC

Lignocellulose degradation in wood and urban-waste composts analysed by functional metagenomics

Nadine Castelhano 1, Hugo Froufe 2, Cristina Barroso 1, 2, Luciana Albuquerque 1, Conceição Egas 1, 2

1 Center for Neuroscience and Cell Biology, University of Coimbra, 3000 Coimbra, Portugal, 2 Next Generation Sequencing Unit, Biocant, 3060 Cantanhede, Portugal

Few metagenomic analyses have been conducted to explore the compost highly efficient microbial system of lignocellulose biomass bio-recycling. These environments are sources of enzymes with high industrial value. The present work aims to compare the lignocellulose degradation capacity between a wood-based compost pile

(COMP18) and an urban-waste composting tower (WR3). Functional metagenome assembly resulted in 19 bins for COMP18 and 41 bins for WR3. The taxonomy analysis identified Acidobacteria as the dominant phylum in COMP18, followed by Chloroflexi and Proteobacteria phyla. On WR3, Firmicutes and Actinobacteria were the most relevant phyla. Two near-complete genomes for COMP18 and nine for WR3 were obtained, in particular Pyrinomonas methylaliphatogenes in COMP18, and Thermobifida fusca for WR3. In order to investigate the functional potential of lignocellulose degradation, the CAZy annotation was investigated by blast searches and manual curation. Only half of the bins of the two composts had lignocellulolytic enzymes for the four substrates studied, cellulose, hemicellulose, pectin and lignin. Pectin degrading enzymes were more abundant on bins of COMP18, and WR3 bins had an important contribution to cellulose, hemicellulose, and lignin degradation. Nevertheless, only a few bins of both metagenomes encompassed enzymes for all types of substrates present on the lignocellulosic biomass. Correlation of the taxonomy and substrate degradation was also done, revealing which phyla were associated with each substrate. A detailed analysis of the identified enzymes can provide valuable information on enzymes for the degradation of lignocellulose with potential application in the biofuel industry.

Funded by projects H2020 Metafluidics - 685474, FCT - UIDB/04539/2020 and GenomePT- POCI-01-0145-FEDER-022184.

Microbiomes of Invertebrates

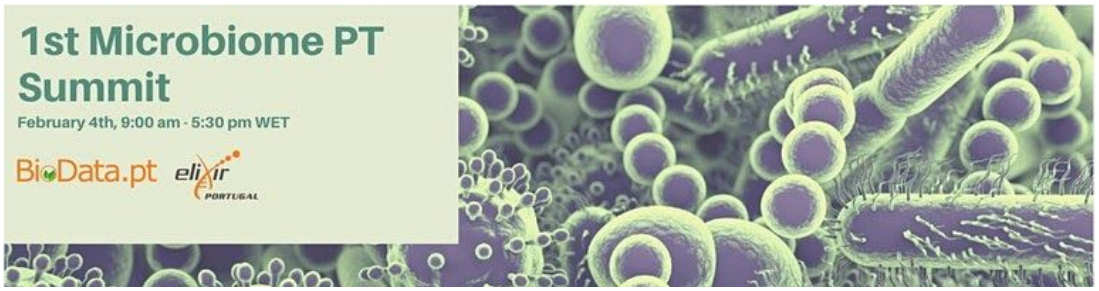
24 - Nelson Martins - Postdoctoral Researcher at Instituto Gulbenkian de Ciência

Genetic bases of Wolbachia driven variation in within-host titers and antiviral

Teresa G. Maia 1, Catarina R. Carmo 1, Nelson E. Martins 1, Luís Teixeira 1

1 Instituto Gulbenkian de Ciência, 2780 Oeiras, Portugal

Wolbachia is a widespread bacterial endosymbiont of invertebrates, known to manipulate the reproduction of its hosts. In some insect hosts, such as the fruit fly Drosophila melanogaster or mosquitoes, Wolbachia provides protection against viral infection. This antiviral effect is currently being applied as tool for arbovirus control by releasing Wolbachia-transinfected mosquitoes in dengue endemic areas. In the Wolbachia strain of D. melanogaster (wMel), within-host symbiont loads show natural variation and this variation correlates with antiviral protection. However, the lack of genetic tools for Wolbachia hampered the characterization of this relationship, and more generally the mechanistic basis of antiviral protection and other aspects of Wolbachia biology. We propose to take advantage of the natural variation of wMel, and, using either published or newly obtained genomic data, will perform a genome wide association study for Wolbachia titers and antiviral protection, to identify the genetic bases for variation in these two traits. These results will serve to pinpoint



candidate gene variants for further functional testing, e.g., as candidates for targeted mutagenesis screens currently being developed in the host laboratory or for ectopic expression in D. melanogaster.