MaCuMBA
PRESENTS

MARINE MICROBIOME
DISCOVERY & INNOVATION

ABSTRACT BOOK

MARINE / BIOTECHNOLOGY / AQUACULTURE / BIOENERGY / PHARMACEUTICALS / FOOD / ENVIRONMENT / COSMETICS / HEALTH

WHERE SCIENCE & INDUSTRY MEET

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AN INDUSTRY CONFERENCE "FACE-TO-FACE" WITH AN OPEN SCIENCE MEETING ON THE BIOTECHNOLOGICAL APPLICATION OF MARINE MICROBES ORGANISED BY MaCuMBA

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ABSTRACT BOOK
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ORAL PRESENTATIONS

Session 1

The diversity of the oceanic microbiome

09:00 – 12:30, Wednesday, 29 June 2016

Location: Bunsen Saal

Chair: Silvia Cretoiu (Royal Netherlands Institute for Sea Research, the Netherlands)

Much debate still persists regarding the extent of the microbial diversity of the oceanic microbiome. This applies to all three domains of life: Eukarya, Bacteria and Archaea. How many species really exist? How are they distributed in the ocean? How many new species are there and where can they be found? Can we find new species with interesting values in extreme marine environments, the deep sea or the deep-sea sediments? Or would the most interesting microbes be symbiotic with marine animals, plants or other microorganisms? This session attempts to give an overview of the present knowledge in relation to these questions.

ORAL PRESENTATIONS - SESSION 1

ACCESSING THE MARINE MICROBIOME THROUGH NOVEL CULTIVATION APPROACHES

Jörg Overmann

DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

09:00 – 09:30, Wednesday, 29 June 2016

With an estimated total number of 6.6×10^{29} cells, Bacteria and Archaea in marine waters and sediments constitute a major fraction of the global microbial biomass. Most marine bacterial communities are highly diverse and may amount to 40,000 species. In contrast, less than 2,000 strains in public culture collections are of marine origin. The persisting difficulty to culture many of the dominant marine bacterial and archaeal phylotypes relates to an insufficient appreciation of the specific physiological requirements and adaptations of marine Bacteria and Archaea. In many marine environments, concentrations of readily utilizable dissolved organic carbon compounds or inorganic nutrients are present at submicromolar concentrations whereas suspended marine particles constitute spatially discrete hot spots of growth substrates. Known bacterial adaptations to oligotrophic growth conditions include high affinity uptake systems, low growth rates and cell sizes, streamlined genomes, little regulatory flexibility, physiological specialization and reduced loss rates due to grazing and viral lysis. On the opposite, lineages adapted to exploitation of nutrient hot spots are motile, chemotactically active, have large cells, adhere to particles, employ specialized uptake systems for high molecular weight substrates, excrete exoenzymes, and feature a broad substrate spectrum. Besides these canonical types of adaptations, interesting novel traits have been discovered over the past years, like the widely distributed proton-pumping bacteriorhodopsins, thiosulfate oxidation, or heavy-metal resistance. Future culture attempts need to be modified according to this improved knowledge of the specific adaptations of marine Bacteria and Archaea. Indeed, some novel cultivation technologies have already allowed isolating a number of novel types of marine bacteria and have enabled novel insights into their ecology and potentials for application.

ORAL PRESENTATIONS - SESSION 1

THE DIVERSITY OF CYANOBACTERIAL METABOLISM

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09:30 – 09:45, Wednesday, 29 June 2016

Cyanobacteria are the only prokaryotic organisms capable of oxygenic photosynthesis. Enabled by their universally available source of energy they form one of the most diverse and widespread phyla. Their habitats range from cold-arctic rocks to 75°C hot springs, from nutrient-poor oceans to fertile rice fields, from rocks to lakes, from animal fur to nuclear power plant wastewater. Where there is even the tiniest amount of light, there is a possible niche for cyanobacteria. With the invention of next-generation sequencing, the availability of fully sequenced genomes throughout the whole tree of life increased significantly, including an increasing number of newly sequenced cyanobacterial strains. Therefore a genomic comparison of cyanobacterial strains is now possible, allowing for a detailed and more significant analysis of the cyanobacterial pan- and metagenome. We describe a comparison of the genomes of 77 fully sequenced cyanobacteria found in the GenBank database. While most studies focus on either genes that can be found in all strains (core-genome), genes unique to single strains, or genes specific to a selected set of stains (signature genes), we focus on the co-occurrence of genes that encode for metabolic enzymes. Genes that cooperatively encode for a specific function can be assumed to occur together in strains where this function is present. By implication, if two genes co-occur in more genomes than expected by chance, they are likely involved in such a common process. In our presentation, we will describe a systematic and unbiased approach to analyse the co-occurrence of all genes across a wide range of cyanobacteria using analysis of genetic cliques. It is shown that co-occurrence indeed correlate with putative functions, and co-occurrence does not necessarily imply proximity on the genome. Our work serves as a starting point for further studies to understanding the complexity and potential of cyanobacterial metabolism across different habitats.

ORAL PRESENTATIONS - SESSION 1

CURACIN-LIKE GENE EXPRESSION AND TOXIN PRODUCTION IN TRICHODESMIUM ERYTHRAEUM

Reut Sorek-Abramovich[a], Hila Elifantz[a], Shmuel Carmeli[b], Ilan[a] Berman-Frank[a]

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09:45- 10:00, Wednesday, 29 June 2016

Trichodesmium spp. is a globally important bloom-forming, marine cyanobacterium that has been found to produce trichotoxin, palytoxin and ciguatoxin-like toxic compounds. It is currently unclear how eutrophication may influence toxin production in *Trichodesmium*. We discovered potential genes relevant to toxin production and examined the toxicity effects of lab cultures towards *Artemia Salina* (brine shrimp). In addition, large scale culturing efforts were made in order to ensure sufficient quantities of dry material for toxin analytical identification. A singular NRPS/PKS gene cluster was found in *T. erythraeum* IMS101 publicly available genome, bearing high homology to the curacin A NRPS/PKS gene cluster from *Moorea producens* (*Lyngbya Majuscula*). Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) primers were then designed for five modules in the *T. erythraeum* NRPS/PKS cluster and an upstream gene, Tery_3815. We examined the impacts of nitrate addition versus N2-fixation on the growth and expression of these specific genes in laboratory cultures. Results indicated that one gene was upregulated, two genes were down-regulated, and three genes did not change significantly relative to control cultures. Laboratory cultures under replete nutrient conditions were grown to typical bloom densities and were then extracted with 70% methanol and separated on an open reversed phase column and HPLC. Thirty-three separated fractions were tested against *Artemia Salina* (brine shrimp). Five hydrophobic fractions caused 60–100% mortality, at final concentrations of 0.1 and 1 mg ml^{-1}. We are currently investigating the target toxin molecules within these fractions. Large scale culturing efforts have proven difficult to master in this relatively slow growing (3-5 days for doubling time) important marine microorganism.

ORAL PRESENTATIONS - SESSION 1

DEEP-SEA FUNGI AS AN UNTAPPED RESERVOIR OF BIOACTIVE MOLECULES

Marion Navarri, Camille Jégou, Benjamin Brillet, Laurence Meslet-Cladière, Georges Barbier, Gaëtan Burgaud and Yannick Fleury

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10:00 – 10:15, Wednesday, 29 June 2016

The recent characterization of a plasmid-mediated colistin resistance has emphasized the critical concern to renew antibiotic arsenal. As natural and natural-derived drugs in pharmaceutical pipelines represent 71% of newly approved molecules (Newman et Cragg 2012), marine habitats have received a special attention as untapped reservoirs of novel bioactive compounds (Blunt et al. 2015). As natural product chemolibraries from marine fungi are rapidly developing along with new reported structures (Rateb et Ebel 2011), we can hypothesize that a close look at fungi from extreme environments, and especially deep subseafloor sediments, will support a dramatic increase of novel active biomolecules in a close future. In the frame of the MaCuMBA European Project, almost 200 deep subseafloor fungal strains isolated from deep sediment samples collected in the Canterbury Basin (New Zealand, IODP Expedition 317) were screened for antimicrobial potential using both genetic and functional approaches. The genome mining using degenerated primers targeting PKS (Polyketide Synthase), NRPS (Non-Ribosomal Peptide Synthetase), hybrids (PKS-NRPS) and TPS (Terpene Synthase) was not discriminative enough since around 97% of deep-sea fungal strains harbor at least one of those genes (Rédou et al. 2015). A functional screening using 16 microbial targets revealed the antimicrobial potential (mainly directed against Gram-positive target bacteria) of 33% of filamentous fungi cultivated on PDA (Navarri et al. 2016). The extraction and analyzes of antimicrobial compounds of the 14 most promising deep subseafloor fungal isolates demonstrated their amphipathic nature and the LC-MS analyses have illustrated their metabolic chemodiversity. The crude extract resulting from *Oidiodendron griseum* strain CB-36 (isolated at 765 meters below seafloor) exhibited an important antimicrobial activity interpreted by the presence of at least four antibacterial compounds. Dihydrosecofuscin was structurally elucidated using NMR and the three other compounds and their biological activities are under investigation.

ORAL PRESENTATIONS - SESSION 1

EVALUATION OF ANTIMICROBIAL POTENTIAL OF THE ANTARCTIC BACTERIUM AEQUORIVITA SP. ISOLATED BY THE MICRODISH CULTURE CHIP

Fortunato Palma Esposito[a], C.J. Ingham[b], D. Tasdemir[c], D. de Pascale[a]

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10:15 – 10:30, Wednesday, 29 June 2016

Seas and oceans contain about 10^9 Bacteria and Archaea and 10^6-10^7 eukaryotic microorganisms per L, comprising an enormous biodiversity. These numbers are in sharp contrast to the ±10.000 microbes that have been described and cultivated. Culturing microorganisms depends on finding the best growth conditions and many times, they are not in line with traditional laboratory approaches. By providing the access to the real biodiversity, of "not-yet-cultivated" or rare microorganisms, the analysis of novel secondary metabolites may lead to a new "Golden Age" of drug discovery with huge benefit for the public health. A very promising strategy for the isolation of new bioactive molecules could be focusing the attention on extreme environments, like Antarctica, in which microbes have accumulated remarkable physiological and functional heterogeneity to survive to harsh life conditions. Moreover, the use of not-conventional isolation techniques could lead to the identification of unknown microorganisms. In this work, new approaches, which mimic the environment by using minimal growth media and the MicroDish Culture Chip (MDCC, developed by MicroDish, The Netherlands) allowed us the isolation of different strains showing antimicrobial activity against a panel of Multidrug Resistant (MDR) bacteria composed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphyloccoccus aureus* and *Acinetobacter baumannii*. One of them belongs to the genus Aequorivita, a Gram-negative Antarctic bacterium, discovered for the first time in 2002 and poorly investigated, shows an inhibition effect against all the target bacteria. Experiments have demonstrated that the compound responsible for the bioactivity is intracellular. A preliminary purification was performed by HPLC and LC-MS. The lack of previous studies about the antimicrobial potential of this bacterium makes it a promising candidate for further investigations.

ORAL PRESENTATIONS - SESSION 1

MICROBIOMICS AT GLOBAL SCALE: FROM THE HUMAN GUT TO TARA OCEANS

Peer Bork

EMBL, Meyerhofstr. 1, 69117 Heidelberg, Germany

11:00 – 11:30, Wednesday, 29 June 2016

Microbiomics that is the scientific study of microbiomes implies quantitative, large-scale research on various ecosystems. While it started in environments such as the ocean more than ten years ago, accelerated funding and size of the research field around the human microbiome led to methodological and conceptual developments that are now being applied to other ecosystems due to multiple analogies. I will review a few milestones and approaches associated with metagenomics of the human gut and will then introduce into some aspects of the TARA oceans project, focusing on prokaryotes. TARA oceans provides major resources of molecular data linked to taxonomic, functional as well as physico- and geochemical measures, but it also generalises or identifies global ecological principles (see Bork et al., Science 2015 and refs therein). For example, while community composition is mostly driven by temperature, population structure follows geographical patterns. Finally, I will introduce into a couple of current projects as TARA oceans is ongoing and it will probably take many years to analyse the data collected so far.

ORAL PRESENTATIONS - SESSION 1

HIGH-THROUGHPUT AMPLICON SEQUENCING REVEALS HIGH FUNGAL DIVERSITY IN NORTH ATLANTIC DRIFTWOOD

Teppo Rämä[a,b], Marie L. Davey[c,d,e], Jenni Nordén[c,f,g], Rune Halvorsen[f], Rakel Blaalid[c], Geir H. Mathiassen[a], Inger G. Alsos[a] and Håvard Kauserud[c]

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11:30 – 11:45, Wednesday, 29 June 2016

Knowledge about the secret life of marine fungi and their journey across the world's oceans in driftwood "boats" dates back to the end of the 1800s. Today wood in temperate and tropical waters is among the most studied marine habitats for fungi, whereas northern driftwood fungi has largely been overlooked. For the first time, we applied high-throughput sequencing on fungi residing in driftwood to reveal the community richness and taxonomic composition in the North Atlantic. Using pyrosequencing of ITS2 amplicons obtained from 49 marine logs, we found 807 fungal operational taxonomic units (OTUs) based on clustering at 97% sequence similarity cut-off level. The total richness was estimated to be 1,400 OTUs. Seventy-four percent of the OTUs had affinity to the phylum Ascomycota, and previously overlooked taxa, such as the genus *Cadophora*, were frequent. One fourth of the OTUs remained unassigned to any fungal class, emphasizing the need for better DNA reference data of marine fungi. Discrepancies between the fungal communities detected by traditional culturing techniques and high-throughput sequencing were found. Our results indicate that oceans host a considerably higher number of fungal species than previously known and that a combination of methods is needed to detect and characterize this diversity.

ORAL PRESENTATIONS - SESSION 1

SEARCHING FOR THE BIOLOGICAL MARINE SOURCES OF LONG CHAIN ALKYL DIOLS

Sergio Balzano, Laura Villanueva, Julie Lattaud, Sebastiaan Rampen, Nicole Bale, Stefan Schouten

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11:45 – 12:00, Wednesday, 29 June 2016

Microorganisms are known to produce a broad variety of lipids, which in some cases are taxon specific. Lipids can be preserved for longer periods than other molecules (e.g. DNA) and in some cases they can be used as biomarkers for the presence of their biological source, both in present and past environments. Modern high throughput sequencing technologies allow a detailed identification of the microbial species present in seawater, and when combined with lipid analyses can provide useful information in linking specific lipid classes to their producers. Culture studies have shown that microalgae from the class Eustigmatophyceae, as well as few other species scattered within the eukaryotic supergroup Heterokontophyta, synthesize long chain alkyl diols (LCD). LCDs consist of a straight chain carbon skeleton with two hydroxyl groups at C-1 and at a mid-chain position, respectively. LCDs can vary in chain length (C_{28} to C_{32}) and in the position of the second hydroxyl group (mainly 13, 14, and 15) and have been often detected in marine particles as well as sediment. However, their distribution differs strongly from that observed in cultures, in particular in the marine environment. To investigate this discrepancy we here combine lipid analyses with 18S rRNA gene amplicon sequencing to infer relationships between LCDs and LCD-producers on suspended particulate matter (SPM) collected from several depths of the photic zone along a transect in the tropical North Atlantic Ocean. LCDs were usually more abundant in surface water compared to the deep chlorophyll maximum (DCM) and higher amounts were present in stations within the influence of the Amazon plume. LCDs were dominated by the $C_{30:0}$ 1,15 diol at all stations and concentrations ranged from 0.2 to 30 ng/L. The 18S rRNA gene data showed that the contribution of Eustigmatophyceae, known to produce the dominant $C_{30:0}$ 1,15 diol, to the total 18S rRNA gene reads was extremely low (< 0.1 % of the reads) in most stations. Other phytoplankton classes which contain LCD-producers were found within our dataset but they accounted for low proportions (< 5%) of our genetic libraries. This is surprising given the relatively high LCD concentration (≈ 10 ng/L) found in the SPM. Laboratory experiments of *Nannochloropsis* spp. Indicate that LCD concentrations are fairly constant at about 20 ft/cell, independently of cultivation conditions. In contrast *Proboscia* diatoms, which are larger sized LCD-producers occasionally blooming in coastal waters, can contain up to 10 pg/cell. Based on this, the amounts of LCDs measured here in the tropical North Atlantic Ocean (10 ng/L) should correspond to a density of small sized LCD-producers of about 0.5 x 10^6 cell/L, or a density of *Proboscia*-like diatoms of 10^3 cell/L. This would imply that 10 to 50% of the phytoplankton sampled from those location were LCD-producers, which is a highly unrealistic scenario given the low contributions of sequences of known LCD-producers. This discrepancy suggests that LCDs are not all derived from living organisms and can persist in the water column

ORAL PRESENTATIONS - SESSION 1

for much longer periods than DNA of LCD-producers. Previous studies have indeed suggested that LCDs are relatively resistant to degradation as they form part of a resistant biopolymer, algaenan, in the outer cell wall of Eustigmatophyceae. In addition, a wider range of microbes than previously thought might be able to synthesize LCDs being responsible for the relatively high concentrations found in the SPM. Future culture experiments should determine whether a larger number of phytoplankton species can produce LCDs, which would improve the ability to couple the dynamics of LCDs and LCD-producers in the water column.

ORAL PRESENTATIONS - SESSION 1

CURRENT AND POTENTIAL EXPLOITATION OF CYANOBACTERIAL NATURAL PRODUCTS IN HEALTH CARE AND BIOTECHNOLOGY INDUSTRY

Ulrike Eberhardt[a], Matthias Neumann[a], Sascha Jung[a], Timo Niedermeyer[a,b], Wolfram Lorenzen[a]

[a] **Cyano Biotech GmbH, Berlin, Germany**

[b] **Eberhard Karls University Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany**

12:00 – 12:15, Wednesday, 29 June 2016

Cyanobacteria have adapted to a remarkable variety of ecological niches over all climate zones of our earth, resulting in an outstanding variety of species. On a molecular level, many cyanobacterial strains are producers of secondary metabolites, which show a wide diversity in their chemical structures and bioactivities[1-4]. But how can we access this intriguingly rich natural resource for the discovery and development of novel lead structures for active ingredients in pharmaceuticals, cosmetics and agrochemicals? In order to make cyanobacterial natural products accessible for lead discovery, we at Cyano Biotech make use of the standardized cultivation of cyanobacteria in photobioreactors from millilitre to hundreds of litres scale, the ability of analytical high performance chromatography in combination with photo diode and mass selective detectors to monitor natural product formation in cell and medium extracts from newly isolated cyanobacterial strains and an isolation process based on preparative centrifugal partition chromatography to isolate cyanobacterial natural products. By continuously applying this combination of methods in a standardized process, we produced a total of about eight thousands of concentration-normalized and with regard to their complexity classified pre-fractionated biomass and medium extracts (PFEs), which are routinely assayed for their antimicrobial activity and readily available for any other bioactivity screening. About 13% of all cyanobacterial PFE fractions show antimicrobial activity. Additionally, we determined the minimal inhibitory concentration and novelty based on their exact mass of around 50 pure compounds in order to determine potential antimicrobial substances from cyanobacteria. In summary, we developed and established a process from the environmental sample over unialgal cultures to standardized pre-fractionated extracts containing potentially novel natural products that can be assayed for bioactivities of commercial interest.

1 Timo Niedermeyer and Mark Brönstrup. 2012. Natural product drug discovery from microalgae. In: *Microalgal Biotechnology: Integration and Economy*, ed. Clemens Posten and Christian Walter, 169-202. De Gruyter.

2 Singh, Rahul Kunwar, Shree Prakash Tiwari, Ashwani K Rai, and Tribhuban M Mohapatra. 2011. Cyanobacteria: an emerging source for drug discovery. *The Journal of Antibiotics* 64: 401-412.

3 Tidgewell, Kevin, Benjamin R Clark, and William H Gerwick. 2010. The Natural Products Chemistry of Cyanobacteria. In: C*omprehensive Natural Products II: Chemistry and Biology,* ed. Lewis Mander and Hung-Wen Liu, 141-188. Elsevier.

4 Sielaff, Heike, Guntram Christiansen, and Torsten Schwecke. 2006. Natural products from cyanobacteria: Exploiting a new source for drug discovery. *IDrugs* 9: 119-127.

ORAL PRESENTATIONS - SESSION 1

DIVERSITY AND BIOACTIVE COMPOUNDS FROM DEEP-SEA ACTINOMYCETES

Fernando de la Calle

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12:15 – 12:30, Wednesday, 29 June 2016

The diversity and drug discovery capabilities of cultivable actinomycetes from deep-sea sediments (350-3000m in depth) have been investigated. After molecular characterization by DNA-fingerprinting and partial amplification of 16S rRNA, a total of 1139 different actinobacteria, belonging to 23 actinobacteria genera, were cultured and their crude extracts tested for antitumor activity, de-replicated by HPLC-MS and purified to isolate new active compounds. Three families of new polyketide/peptides were elucidated as well as more than twenty other families of cytotoxic compounds. This research has been developed during the MaCuMBA project and it demonstrates the enormous potential of deep sea actinobacteria as a source of polyketide and non-peptide bioactive compounds.

ORAL PRESENTATIONS

Session 2

Novel (meta)genomic approaches in marine microbiology

13:30 – 17:00, Wednesday, 29 June 2016

Location: Bunsen Saal

Chair: Francisco Rodriguez Valera (Universidad Miguel Hernandez, San Juan de Alicante, Spain)

Next Generation (high throughput) Sequencing (NGS) presents unprecedented possibilities for uncovering the marine microbiome. It allows for the reconstruction of metabolic pathways and uncovering of metabolic networks. Metagenomic (including transcriptomics, proteomics, metabolomics) techniques will infer novel functions and it may inform strategies for how to isolate and grow novel marine microbes. These techniques are so precise they allow for investigation even on a single cell level. Metagenomics can be used as an approach for targeted isolation of microorganisms with interesting properties. It can also uncover the diversity and distribution of secondary metabolites. Cloning and expression of genes in the (meta)genomes can be used to find novel products. This session will discuss state-of-the-art techniques in this field and present an outlook on the possibilities and impossibilities of these approaches to understand the function of the marine microbiome and how to mine it for novel applications.

ORAL PRESENTATIONS - SESSION 2

INTEGRATED (META) GENOMIC AND SYNTHETIC BIOLOGY APPROACHES FOR BIOACTIVE BIODISCOVERY

F. Jerry Reen[a] and Fergal O'Gara[b]

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13:30 – 14:00, Wednesday, 29 June 2016

In recent years, the marine environment has been the subject of increasing attention from biotechnological and pharmaceutical industries as a valuable and promising source of novel bioactive compounds. Marine biodiscovery programmes have begun to reveal the extent of novel compounds encoded within the enormous bacterial richness and diversity of the marine ecosystem. A combination of unique physicochemical properties and spatial niche-specific substrates, in wide-ranging and extreme habitats, underscores the potential of the marine environment to deliver on functionally novel bioactivities. The translational benefit of harnessing the technological advances in metagenomics based research promises a new horizon for biodiscovery research. The explosion in genomics based sequence analysis, allied with parallel developments in synthetic and molecular biology, has the potential to fast-track the discovery and subsequent improvement of a new generation of bioactive compounds. Through metagenomics and culture based discovery we have uncovered small molecule signal mimics that neutralise toxin production, biofilm formation, and virulence in clinical pathogens, such as the recently uncovered novel coumarin-like class. Using synthetic chemistry to decorate the alkylquinolone signal molecule framework has enabled us to expand the activity of lead compounds for control of virulence in fungal and yeast pathogens. Another emerging approach is the use of green chemistry to diversify small molecules for the development of innovative molecular therapeutics towards cancer and infection. With the growing need for green alternatives to industrial processes, and the unique transformations which nature is capable of performing, marine biocatalysts are themselves being pursued for their potential to markedly improve current industrial pipelines. Biocatalysts are known to possess chiral selectivity and specificity, a key focus of pharmaceutical drug design. Metagenomics guided discovery of marine and soil samples has identified novel 'first-in-class' biocatalysts which we are further developing through molecular and synthetic biology approaches. Integrated (meta)genomics based biodiscovery is uniquely placed to deliver on the promise of the marine and other ecosystems, providing unheralded access to the untapped potential that has yet to be awakened.

ORAL PRESENTATIONS - SESSION 2

METAGENOMIC APPROACH TO SULFATE-DEPENDENT METHANE OXIDATION

Jon S. Graf[a], Jana Milucka[a], Timothy G. Ferdelman[a], Boran Kartal[b] and Marcel M.M. Kuypers[a]

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14:00 - 14:15, Wednesday, 29 June 2016

Sulfate reduction-coupled anaerobic oxidation of methane (AOM) is a major sink of methane in the ocean and plays an important role in the sedimentary biogeochemical cycling of carbon and sulfur[1]. The AOM process is mediated by methanotrophic archaea (ANME) and associated *Deltaproteobacteria* belonging to the *Desulfosarcina/Desulfococcus* (DSS) clade. Two mechanisms for AOM have been recently proposed which both credit ANME for methane oxidation. One hypothesis suggests that sulfate reduction is performed by the DSS using electrons that are directly transferred from the archaea[2,3]. The other hypothesis postulates that ANME also perform sulfate reduction and that the resulting zero-valent sulfur species are disproportionated to sulfide and sulfate by the DSS[4]. We tested current AOM hypotheses using a comprehensive data set encompassing metagenomic, metatranscriptomic and shotgun metaproteomic information gathered from a highly enriched AOM culture performing sulfate-dependent AOM. We have obtained two nearly complete draft genomes belonging to ANME-2c and DSS (SEEP-SRB1), identified by full-length 16S rRNA gene present in the two genomic bins. We were able to retrieve all necessary genes for methane oxidation via the reverse methanogenesis pathway in the ANME draft genome and could confirm expression of these genes on both transcript and protein level. Within the DSS draft genome we found high expression of all core genes of the canonical sulfate reduction pathway (*Sat*, *Apr*AB, *Dsr*AB) as well as genes encoding for putative accessory proteins for sulfate reduction (e.g. *Dsr*C, *Dsr*MKJOP, *Qmo*ABC). We speculate that these DSS genes might be involved in sulfur disproportionation (as it has been shown for other disproportionating bacteria[5]) and propose that the initial disproportionation of zero-valent sulfur to sulfite and sulfide could be mediated by a yet-unidentified periplasmic enzyme. We further screened our datasets for the presence and expression of genes with a potential involvement in the postulated interspecies electron or metabolite transfer. These involved *c*-type cytochromes, pili and known and hypothetical sulfur-metabolizing enzymes. While pieces of the AOM puzzle are still missing, our data provide support for the previously proposed AOM mechanism of archaeal sulfate reduction and bacterial sulfur disproportionation.

1 Reeburgh WS (2007) Chem. Rev. 107(2), 486 - 513
2 McGlynn S et al. (2015) Nature 526, 531-535
3 Wegener G et al. (2015) Nature 526, 587-590
4 Milucka J et al. (2012) Nature 491, 541-546
5 Frederiksen T et al. (2003) Biodegradation 14.3, 189-198

ORAL PRESENTATIONS - SESSION 2

FROM SIMPLE TO COMPLEX DEGRADATION OF HYDROCARBONS. A CONCEPT OF METAGENOME SUCCESSION IN OIL-AMENDED MICROCOSMS

Deni Ribicic[a], Odd Gunnar Brakstad[b], Roman Netzer[b], Terry C.Hazen[c], Finn Drabløs[a]

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14:15 - 14:30, Wednesday, 29 June 2016

Many groups of microbes exhibit degradation capabilities of different hydrocarbon compounds in the ocean. Oil degradation capabilities are potentially expressed from genes through functional enzymes. Some of the microbes are strictly hydrocarbon specialist, whilst many others are able to consume wider ranges of substrates. Here, we introduce the concept of metagenome succession as analogy to the microbial community succession in an oil-contaminated environment. The concept itself relies on the general principle of ecological succession which includes the development of a simple to a more complex structure. In the case of metagenome succession, genes encoding for enzymes relevant for degradation of simple hydrocarbons are successively substituted with genes encoding for enzymes potentially involved in degradation of more complex hydrocarbon substrate during microcosm biodegradation of chemically dispersed oil (median oil droplet size 10μm) in cold seawater (5°C). The pattern we could observe involved successions of genes coding for alkane monooxygenase, followed by haloalkane dehaloganes genes in degradation of simple n-alkanes ($<nC_{20}$). Thereafter, phenol hydroxylase, catechol dioxygenase and biphenyl dioxygenase encoding genes, degrading more complex alkanes ($>nC_{25}$), aromatics and PAHs peaked in abundance. The taxa contributing the most to the abundance of respective genes were found within the class of *Gammaproteobacteria*, specifically among the orders *Oceanospirillales, Alteromonadales* and *Pseudomonadales*. In addition the classes of *Flavobacteriia*, *Alpha-* and *Beta- proteobacteria* contributed as well to the respective gene abundance. The microbial community and metagenome compositions in our microcosms exhibited high potential for oil biodegradation under the tested conditions. The data obtained in this study may improve our information on how microbial communities and metagenomes may be associated with oil compound biodegradation when dispersants are used as an oil spill response (OSR) tool in cold environments. The information provided may also be used for oil fate and exposure models (e.g. the OSCAR model) for the prediction of the fate of the oil after an oil spill, and which are used for contingency planning.

ORAL PRESENTATIONS - SESSION 2

A NEW PIPELINE FOR GENUS TARGETED ENVIRONMENTAL SAMPLING BRINGS DEEPER INSIGHTS INTO THE LIFESTYLE AND HABITAT OF MARINE SYNECHOCOCCUS

Frances Pitt[a], Andrew Millard[a], Monica Moniz[b], Martin Ostrowski[c], Laurence Garczarek[b], David Scanlan[a]

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[c] Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia 2109

14:30 - 14:45, Wednesday, 29 June 2016

Cyanobacteria of the genus *Synechococcus* are a ubiquitous component of the oceanic photosynthetic community. Alongside clade targeted culturing efforts, a conserved marker gene analysis has revealed spatial partitioning of locally adapted, genetically distinct lineages across seasons, ocean basins and biomes. To forward our understanding of the molecular basis for this niche adaptation and thus inform new cultivation techniques we have undertaken a two stage targeted molecular approach. Our strategy combines exclusive flow cytometric sorting of natural *Synechococcus* populations from the Atlantic Ocean, with both metagenomic and transcriptomic high throughput sequencing. Rigorous pipeline trials with *Synechococcus* sp. BL107 demonstrate that water filtration has a well-defined, limited impact on the transcriptomic profile and that sample storage and multiple rounds of cell sorting have almost no effect on the RNA profile. This targeted approach aims to identify clade specific gene sets, with the opportunity to discover those actively being expressed across different geographic provinces. This approach will help to explore the role that specific gene sets play in defining the ecological 'distinctness' of these lineages, giving new insights into how we can better tailor future cultivation strategies.

ORAL PRESENTATIONS - SESSION 2

DESCRIPTION OF MICROBIAL DIVERSITY AT A TIDAL HYDROTHERMAL VENT SITE BY CULTIVATION AND NEXT GENERATION SEQUENCING (NGS)

Pauline Vannier, Alexandra Klonowski, Eyjólfur Reynisson, Gregory Farrant, Pauline Bergsten, René Groben, Sigurlaug Skírnisdóttir, Justine Vanhalst & Viggó Þór Marteinsson

Matís ohf./Food Safety, Environment and Genetics, Vínlandsleið 12, 113 Reykjavík, Iceland

14:45 - 15:00, Wednesday, 29 June 2016

Reykjanes peninsula (Westfjords, Iceland) contains tidal hydrothermal vent sites where hot springs are located at the intertidal zone and are submerged in sea water at high tide but exposed at low tide. This creates a unique environment where microorganisms are subjected to vastly different conditions on a diurnal rhythm, e.g. temperatures between 4°C and 85°C. Water samples were taken at locations varying in the influx of hot freshwater from the geothermal springs and of cold sea water during high tides. In addition, in situ enrichments on dried seaweed were placed at those locations and retrieved after three months deployment. Molecular taxonomic analyses of the samples were carried out by high-throughput Next Generation Sequencing (NGS) of 16S rRNA genes. In addition, metagenomic sequencing was done to access the variety of metabolic pathways and genes. Taxonomic analyses showed rather low biodiversity and patterns that were characterized by temperature fluctuation. Differences in the dominance of various taxa could be observed among samples, but well known thermophilic genera, like *Thermus*, *Rhodothermus*, etc. occurred in all samples at high abundance. Other non-thermophilic taxa, like *Rheinheimera*, *Pseudomonas*, etc. were observed as well. Characterization of metagenomes confirmed the taxonomic analysis, however, sequences with low identities to genes from known taxa were also identified, most likely representing novel prokaryotic species and genes. More than 300 strains were isolated by plating on solid medium, dilution-to-extinction, MicroDish®, cell sorting and with an artificial support approach. Isolated strains were characterized by sequencing and MALDI-ToF analysis to confirm their identity. Many of the represented taxa, especially the dominant ones, were successfully cultivated. Stimulating biofilms on artificial supports was the most successful method to cultivate some of the rarer species. The cultivation confirmed the findings of the NGS analysis of an overall low diversity in the samples with a few dominant taxa that were primarily isolated. Rarer taxa were present but required special techniques for isolation and cultivation. NGS data helped in shaping them as knowledge of expected taxa and their requirements allowed for a more targeted approach in isolation and cultivation. This showed the successful combination of culture- and non-culture-based methods in the characterization of microbial biodiversity in an extreme environment.

ORAL PRESENTATIONS - SESSION 2

TARGETING TAXONOMIC BLIND SPOTS AND FUNCTIONAL TRAITS VIA SINGLE-CELL APPROACHES

Tanja Woyke

DOE Joint Genome Institute, Walnut Creek, CA, USA

15:30 - 16:00, Wednesday, 29 June 2016

Genomic sequencing of bacterial and archaeal genomes has revolutionized our understanding of microbial evolutionary diversification and metabolic capacity, and significantly sped up discoveries made in bioprospecting. While we are soon approaching the 100,000 mark for bacterial and archaeal sequenced genomes worldwide, the bulk of them are of rather limited phylogenetic diversity, as the microbial tree of life is dominated by major branches with no cultivated representatives. Many of these branches had even eluded detection in cultivation-independent environmental PCR surveys due to PCR primer mismatches and amplification biases caused by intervening sequences in 16S rRNA genes, leaving major so-called taxonomic blind spots. This talk will discuss the utility of single-cell approaches to access genomes of such taxonomic blind spots and exemplify the emergence of targeted single-cell approaches that aim to enrich organisms and genomes with functional traits of interest.

ORAL PRESENTATIONS - SESSION 2

DIVERSITY, EVOLUTION AND GLOBAL DISTRIBUTION OF MARINE SYNECHOCOCCUS PIGMENT TYPES (CYANOBACTERIA)

Frédéric Partensky[a*], Théophile Grébert[a], Hugo Doré[a], Gregory K. Farrant[a], Joe San Filippo[b], David M. Kehoe[b], David M. Scanlan[c], Silvia Acinas[d] & Laurence Garczarek[a]

[a] Station Biologique de Roscoff, UMR 7144 CNRS & Université Paris VI, Marine Phototrophic Prokaryotes (MaPP) Team, CS 90074, 29688 Roscoff, France

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[*] Presenting author: frederic.partensky@sb-roscoff.fr.

16:00 - 16:15, Wednesday, 29 June 2016

Synechococcus is one of the two most abundant marine oxyphototrophs, accounting for more than 15% of global oceanic primary production[1,2]. This ubiquitous marine picocyanobacterium possesses large extrinsic light-harvesting complexes called phycobilisomes (PBS) that display a wide range of absorption properties due to the variability of their constitutive phycobiliproteins and covalently bound pigments[3]. These sophisticated antenna complexes are mainly encoded by a specific genomic region (PBS region) ranging from 9 to 28 kbp in length and constituted of genes that have been laterally transferred among lineages during the evolution of the *Synechococcus* genus[3,4]. Three main pigment types (1-3) and five subtypes (3a-e) have been defined based on their distinct spectral characteristics, each being associated to a specific PBS gene complement and organization of the PBS region. Chromatic acclimaters (3d-e) possess an additional gene island[3,4] and the genetic characterization of two transcriptional regulators located in this island and showing diametric effects on their regulon has brought new insights into this phenomenon[5]. Genetic markers specific of each pigment type were designed and used to recruit reads from the Tara Oceans metagenome data set. These reads were then assigned to the corresponding pigment type by phylogenetic placement on reference trees. This original approach allowed us for the first time to assess the global distribution of *Synechococcus* pigment types in the world ocean.

1 Flombaum P et al. (2013). Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. PNAS 110: 9824-9829.

2 Farrant GK, Doré D, Cornejo-Castillo FM, Partensky F, [7 authors] & Garczarek L (2016). Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria. *PNAS Plus*. In revision.

3 Six C et al. (2007). Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. *Genome Biol* 8: R259.

4 Humily F, Partensky F, Six C, Farrant GK, Ratin M, Marie D & Garczarek L. (2013). A gene island with two possible configurations is involved in chromatic acclimation in marine *Synechococcus*. *PLoS One* 8: e84459.

5 Sanfilippo J, Nguyen A, Karty J, Shukla A, Schluchter W, Garczarek L, Partensky F & Kehoe DM (2016). A self-regulating genomic island encoding tandem regulators confers chromatic acclimation to marine *Synechococcus*. *PNAS*. In press.

ORAL PRESENTATIONS - SESSION 2

BOTTLE EFFECT IN MARINE METAGENOMES

Mario López-Pérez, José M. Haro-Moreno and Francisco Rodriguez-Valera

Universidad Miguel Hernandez, San Juan de Alicante, Spain

16:15 – 16:30, Wednesday, 29 June 2016

Community response dynamics across short time scales of incubations are poorly understood, sometimes the reliability of conclusions drawn from natural metagenomes can be compromised by artifacts of confinement ("bottle effects"). Here, we have used direct metagenomics sequencing to explore temporal successions of microbial populations in response to different periods of incubations (7 and 14 hours) of seawater from different depths (15, 60 and 90 meters) and placed in darkness and room temperature. Incubations were conducted in the Mediterranean Sea during early autumn, when the water column is stratified.

ORAL PRESENTATIONS - SESSION 2

A TARGETED MUTATION SYSTEM IS ACTIVE IN THE FILAMENTOUS N2-FIXING CYANOBACTERIA TRICHODESMIUM ERYTHRAEUM

Ulrike Pfreundt, Wolfgang R. Hess

Faculty of Biology, University of Freiburg, Schaenzlestr. 1, 79104 Freiburg, Germany, ulrike.pfreundt@biologie.uni-freiburg.de

16:30 - 16:45, Wednesday, 29 June 2016

Photosynthetic cyanobacteria of the genus *Trichodesmium* are responsible for 30-60% of global marine nitrogen fixation, and thrive in oligotrophic waters. Strongly deviating from other bacteria with oligotrophic lifestyle, only 64% of the *Trichodesmium erythraeum* IMS101 genome codes for protein (~85% in other sequenced bacterial genomes). Using primary transcriptome sequencing, we demonstrated that *T. erythraeum* has the highest percentage of transcription start sites for non-coding RNAs of any bacterium examined to date (Pfreundt et al., 2014). Up to 86% of the intergenic sequence space is transcribed into RNA in culture and the sequence highly conserved in metagenomics datasets of *Trichodesmium* (Walworth et al., 2015). We further found a sequentially splicing group II twintron (nested introns, Pfreundt and Hess, 2015), the only twintron known to interrupt a protein-coding gene in bacteria, raising the question about possible special adaptations of the *Trichodesmium* genome to mobile genetic elements. This idea is in line with our finding of a highly transcribed retroelement (Diversity Generating Retroelement, DGR) that serves as a template for targeted mutation of at least 12 genes by mutagenic homing (Pfreundt et al., 2014). Using individually picked *Trichodesmium* colonies from the Gulf of Mexico and amplicon sequencing, we have generated first evidence that some of the putative target genes are indeed highly diverse even within few individual *Trichodesmium* colonies, indicating high mutation rates at these genomic sites. While there is no functional annotation for these genes, a role in viral defense, anticipatory ligand binding, or cell-to-cell interactions can be imagined. This bacterial DGR has immense potential for the engineering of targeted massive protein diversification in vivo.

ORAL PRESENTATIONS - SESSION 2

ANALYSIS OF MICROBIAL COMMUNITY COMPOSITION OF SEDIMENTS FROM THE SOUTHERN GULF OF MEXICO

M Asunción Lago-Lestón, Dante Magdaleno-Moncayo, Briceida Covarrubias and Lluvia Vargas-Gastélum

CICESE, Carretera Tijuana-Ensenada 3918, Ensenada 22860, Baja California, México. alago@cicese.mx

16:45 - 17:00, Wednesday, 29 June 2016

Marine sediments are home to highly abundant and diverse microbial communities, however, due to its vastness, this environment still remains poorly studied. The Gulf of Mexico is an ocean basin characterized by both shallow waters and deep-water areas and can reach depths up to 4,000 m. Although, there are some studies describing biological diversity in the Gulf, very few of them involve microbial communities in the sediments, particularly from deep areas. Furthermore, not until recently have appeared some studies about microbial assembles of Gulf sediments but as a consequence of the accidental Deepwater Horizon oil spill but these are mostly focused in the Northern American region of the Gulf, not in the Mexican economic exclusive zone. In order to establish a baseline of the diversity of microorganisms living in the sediments of the Southern Gulf of Mexico and to shed some light into the unknown microbial communities of the area, a sampling campaign (XIXIMI 3) was conducted during February - March of 2013. Here, we present results from five different sediment samples collected at different depths among 1500 to 3500 meters. DNA was extracted and metagenomic shotgun libraries were prepared and sequenced using the Illumina MiSeq technology. Unassembled reads were annotated taxonomically using MG-RAST and a customized QIIME pipeline. Results showed differences among samples and pipelines. At phylum level composition, Proteobacteria appears as the most abundant group (until ~70% in absolute values), mainly represented by Alpha and Gammaproteobacteria (~ 30% each) when MG-RAST pipeline was used. These percentages are lower when QIIME pipeline was run. Acidobacteria and Planctomycetes followed as ones of the most abundant phyla in both analyses. On the contrary, other groups, such as Firmicutes, appear highly represented when data was obtained through MG-RAST, but only represents at maximum of 3% if QIIME was used. Most changes among samples were observed at the class level, where classes such as Bacteroidetes, Acidobacteria and Firmicutes present different abundances among the different sites. Archaea represents 20% of the total of prokaryotes, mainly composed by Thaumarchaeota and Euryarqueota, but clear changes among sites where mostly found in Nanoarchaeota group, showing a higher abundance in one of the deeper sampling sites. This result could be suggested the presence of either methane seeps or hydrocarbon leakage at that point.

ORAL PRESENTATIONS

Session 3

Marine systems microbial ecology

09:00 – 12:30, Thursday, 30 June 2016
Location: Bunsen Saal

Chair: Lone Gram (Technical University of Denmark, Kgs. Lyngby, Denmark)

Marine microbial systems should be studied as functional entities formed by the microbial diversity and the biogeochemical cycles they encompass. In this systems ecology approach the network of different microorganisms, and the way they communicate, can be studied by using meta-omics (metabolomics) and numerical modelling. This session aims at bringing together the newest developments in systems microbial ecology.

ORAL PRESENTATIONS - SESSION 3

ECOGENOMICS OF MARINE MICROBIAL COMMUNITIES

Gerard Muyzer

Microbial Systems Ecology, Department of Aquatic Microbiology, Institute for biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands

09:00 - 09:30, Thursday, 30 June 2016

Next-generation sequencing of DNA has revolutionized microbial ecology. By using this technology, it became for the first time possible to analyze hundreds of samples simultaneously and in great detail. 16S rRNA amplicon sequencing, metagenomics and metatranscriptomics became available to determine diversity and activity of microbial communities. Moreover, the huge amount of data that is obtained made it possible to build statistically significant networks from which ecological (or metabolic) interactions amongst microbes and between microbes and their environment could be inferred. Here I give examples on the application of next-generation sequencing to characterize marine microbial communities.

ORAL PRESENTATIONS - SESSION 3

CULTIVATION, INTERACTIONS AND BIOMOLECULES OF MARINE SYNECHOCOCCUS

Alicja Dabrowska, Despoina Sousoni, Blanca Perez Sepulveda, Frances Pitt, Monica Moniz, Christophe Corre, Joseph Christie-Oleza, David J. Scanlan

School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL UK (Partner 13)

09:30 - 09:45, Thursday, 30 June 2016

Synechococcus is a ubiquitous member of the marine picophytoplankton community playing a major role in oceanic carbon cycling. We are pursuing new approaches to isolate into culture various marine *Synechococcus* lineages known from environmental sequencing of the high-resolution marker petB [1]. One approach involves the use of 'helper' bacteria to facilitate higher plating efficiencies and longer term culture stability. However, there is a dearth of mechanistic information on interactions between *Synechococcus* and heterotrophic organisms co-occurring in oceanic waters. We have been studying these interactions using *Roseobacter* as the model heterotroph (a dominant and widespread member of the bacterioplankton community)[2, 3]. An exometabolomic analysis showed that *Synechococcus* strain WH7803 produces a molecule in high abundance only when the culture is axenic and entering stationary phase (prior to entering its death phase). This metabolite has been purified and is currently undergoing high resolution NMR analysis to solve the structure.

1 Mazard et al., (2012) Multi-locus sequence analysis, taxonomic resolution and biogeography of marine *Synechococcus*. Env. Microbiol. 14: 372-386.

2 Christie-Oleza et al., (2015) Functional distinctness in the exoproteomes of marine *Synechococcus*. Env. Microbiol. 17: 3781-3794.

3 Christie-Oleza et al., (2015) "You produce while I clean up," a strategy revealed by exoproteomics during *Synechococcus–Roseobacter* interactions. Proteomics 15: 3454-3462.

ORAL PRESENTATIONS - SESSION 3

INSIGHTS INTO THE DIVERSITY, PHYSIOLOGY, FUNCTIONS, ECOLOGICAL ROLES AND BIOTECHNOLOGICAL POTENTIAL OF DEEP SUBSEAFLOOR MARINE FUNGI USING AN INTEGRATED APPROACH

Burgaud G., Rédou V., Navarri M., Jégou C., Brillet B., Meslet-Cladière L., Fleury Y. & Barbier G.

Université de Brest, EA 3882, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB, Technopôle de Brest Iroise, 29280 Plouzané, France

09:45 - 10:00, Thursday, 30 June 2016

Bacteria and Archaea are the most commonly studied microorganisms in the marine environment, and habitats such as deep subseafloor ecosystems are no exception. However, recent studies strongly support the idea that deep subseafloor microbial communities include Fungi, which seem to dominate those micro-eukaryotic communities. Using sediment samples from the IODP Expedition 317 as a model, our aims were (i) to better understand the diversity, physiology and functions of deep subseafloor fungi, (ii) to provide clues about how they interact with other microbial populations in those communities and (iii) to assess their biotechnological potential. Using a record depth sediment core, fungal molecular signatures and fungal cultures were obtained from samples as deep as 1740mbsf (Rédou et al. 2014) and 1884mbsf (Rédou et al. 2015), respectively. In spite of the fact that those complementary approaches revealed low diversity of higher fungal lineages, DNA and rRNA signatures as well as almost 200 cultured isolates (harvested using cultivation systems mimicking their natural habitats) provide direct evidence that fungi persist in this challenging ecosystem. Consistent with this idea, physiological analyses indicate some deep subseafloor fungal isolates appear well-adapted to in situ conditions. Metatranscriptome analysis provided an examination of the functional repertoire of deep subseafloor fungi. Gene expression was assigned to metabolic and biosynthetic processes, responses to stress, cell and membrane functions, conidiogenesis and biosynthesis of secondary metabolites (Pachiadaki et al., in revision). These results all provide further support for the notion of fungal presence and activity in the deep subseafloor biosphere, with the ability to interact with other microbial populations by synthesizing antimicrobial compounds (Navarri et al. 2016).

ORAL PRESENTATIONS - SESSION 3

DEEP SEA HYDROTHERMAL VENTS: DIVERSITY OF PIEZOPHILES AND ADAPTATION TO HIGH HYDROSTATIC PRESSURE

Mohamed Jebbar

Université de Bretagne Occidentale, CNRS, Ifremer, UMR 6197-Laboratoire de Microbiologie des Environnements Extrêmes (LM2E), Institut Universitaire Européen de la Mer (IUEM), 4 rue Dumont d'Urville, 29 280 Plouzané, France

10:00 - 10:15, Thursday, 30 June 2016

The deep biosphere (continental underground and in oceans below 1000 m in depth) could represent up to 70% of all cells on Earth, as well as 50% of the primary production of biomass. The deep sea is characterized not only by high pressures (up to 110 MPa) but also by a lack of sunlight, an extremely low temperature (<5°C) except in the vicinity of hydrothermal vents, where temperature may be as high as 460°C, but water remains liquid owing to the high hydrostatic pressure (HHP). Recent trends and advances in molecular ecology, metagenomics, etc give evidence that microorganisms (*Bacteria, Archaea*, Viruses, Fungi and Protists) represent by far the most important biological group on deep sea in terms of phylogenetic and functional diversity. The enormous diversity of deep sea microorganisms also gives rise to a largely untapped reservoir of genetic information, bioactive compounds and biomolecules (e.g. enzymes) which may find an application in various domains. Since the discovery of deep-sea hydrothermal vents, many mesophilic and hyper/thermophilic *Bacteria* and *Archaea* have been described but only a few thermo-piezophilic belonging mainly to the *Thermococcales* group have been described so far. The genome data mining showed no obvious general piezophilic signatures and the data related to genome expression (transcriptomic and proteomic studies) were performed by comparing piezophilic (*Thermococcus barophilus, Pyrococcus yayanosii*) versus non piezophilic (*Thermococcus kodakarensis, Pyrococcus furiosus*) species have highlighted the importance of several gene clusters and metabolic pathways, such as energy production and conversion, in the adaptation to HHP. Genetic manipulations were performed in *T. barophilus* in order to investigate the functions of some above pathways in vivo and to examine the roles of related enzymes. This will provide greater insight into the piezophilic lifestyle of thermo-piezophiles microorganisms dwelling in deep biosphere.

ORAL PRESENTATIONS - SESSION 3

SYMBIOSES BETWEEN NITROGEN-FIXING CYANOBACTERIA AND HAPTOPHYTE MICRO-ALGAE

Catherine Ribeiro[a], Adriana Lopes[b], Daniel Vaulot[b]

[a] **Oceanographic Institute, Universidade de São Paulo, São Paulo, Brazil**
[b] **UPMC (Paris-06) and CNRS, UMR 7144, Station Biologique, Place G. Tessier 29680 Roscoff, France**

10:15 - 10:30, Thursday, 30 June 2016

About four years ago, a new type of oceanic symbiosis has been discovered, associating a very small nitrogen-fixing cyanobacterium (UCYN-A) and a picoplanktonic micro-alga, belonging to the Haptophyta division, more specifically to family Braarudosphaeraceae. The cyanobacterium is unable to fix carbon but provides fixed nitrogen to its host, which in turn supplies it with carbon fixed during photosynthesis. Genetic and genomic analyses have suggested that at least three types of UCYN-A and two different hosts exist. Neither the cyanobacteria nor their hosts have been yet isolated in culture. Recent reports have suggested that nitrogen fixation to be specifically important on the continental shelf off Brazil. Using samples collected during the CARBOM cruise in late 2013 in this region, we have sorted natural pico- and nano-planktonic population of eukaryotes by flow cytometry and sequenced using High Throughput Sequencing (HTS) three genes on each population: nuclear 18S, plastid 16S and *nifH* involved in nitrogen fixation. The data confirm the importance of UCYN-A/Braarudosphaeraceae symbiosis off Brazil and allow us to determine more precisely the relationships between the different hosts and symbionts.

ORAL PRESENTATIONS - SESSION 3

SINGLE-CELL ANALYSIS OF MICROBIAL ACTIVITY IN BENTHIC MARINE ENVIRONMENTS

Anne Dekas

Department of Earth System Science, Stanford University, CA, USA

11:00 - 11:30, Thursday, 30 June 2016

Deep-sea sediments cover approximately two-thirds of the planet, and are densely packed with diverse microbial cells. Many of these cells mediate metabolisms relevant to the global climate, including the consumption and production of methane and carbon dioxide. However, due to difficulties accessing and culturing deep-sea microorganisms, the deep sea remains one of the least well-characterized microbial habitats on Earth. Understanding the activity and metabolic capabilities of uncultured benthic marine microorganisms is a key to understanding benthic ecosystems and their role in biogeochemical cycling and climate. In this talk, I will describe the use of nanoscale secondary ion mass spectrometry (nanoSIMS) to measure the activity of single bacterial and archaeal cells within complex environmental samples, and share recent results on microbial activity within deep-sea methane seeps, whale-falls, and representative deep-sea sediment.

ORAL PRESENTATIONS - SESSION 3

MICROBIOMES OF MACROPHYTES IN THE MARINE REALM ACROSS SPECIES, TISSUES, LATITUDES AND ENVIRONMENTAL CONDITIONS.

Aschwin H. Engelen[a], Tânia Aires[a], Gerard Muyzer[b], Ester A. Serrão[a]

a Centro de Ciências do Mar (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

b Microbial Systems Ecology, Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands.

11:30 - 11:45, Thursday, 30 June 2016

It is becoming increasingly clear that the microbiomes of marine and terrestrial organisms can add genetic diversity and functions that play important roles in host development and ecology. Although the characterization of these communities associated with seaweeds are becoming more common, the drivers and functional roles of these associated microbes is still hardly explored. In terrestrial plants, the associated microbiome performs essential functions for their hosts, such as providing nutrients, defense and growth stimuli. However, another part of that same community also can affect the host negatively by outcompeting beneficial bacteria, causing disease and/or degrading plant products and tissue. In contrast to terrestrial plants little is know about microbiomes associated with marine macrophytes. Even though they have to cope with and defend themselves against a high biofouling pressure on a constant basis. By elucidating bacterial community structure, composition and function associated with seaweeds and seagrasses across a multitude of factors and conditions, we hope to contribute to innovative insights in marine microbiomes and their interaction with their hosts. The analysed seaweed microbiomes show a strong species specificity component in their composition and structure as well as an environmental component linked to the occupied habitat of their algal host. Intraspecific differentiation of associated microbiomes was evident between haploid and diploid stages of the heteromorphic biphasic seaweed *Asparagopsis armata*, whereas a genetically uniform invasive brown seaweed *Sargassum muticum* showed latitudinal microbiome clines. This latitudinal trend increased from basal to apical tissues. Experimental ocean acidification had very limited effects on seaweed and the gut microbiome of a grazing isopod. However, food and gut microbiome strongly differed in their bacterial community indicating that this marine grazer is not what it eats. In contrast, addition of seaweed and nutrients strongly, but differently, affected the bacterial communities of intertidal sand flats and seagrass rhizospheres. We conclude that the microbiomes of marine macrophytes have a strong host as well as environmentally driven components. More information on the hologenomes is required to elucidate intertwined relationships within these holobionts.

ORAL PRESENTATIONS - SESSION 3

DISCOVERY OF NOVEL BIOACTIVE COMPOUNDS THROUGH SYNTHETIC ECOLOGY

Henk Bolhuis

Royal Netherlands Institute for Sea Research, NIOZ, and Utrecht University, POBox 140, 4400 AC Yerseke Henk.bolhuis@nioz.nl

11:45 - 12:00, Thursday, 30 June 2016

Screening for novel bioactive compounds for use in biotechnological or pharmaceutical applications often depends on the analysis of single microbial isolates. This approach is hampered by the inability to isolate the majority of extant microbial species into pure culture whereas molecular approaches on high diversity communities at best offer fragmented datasets. One of the problems in microbial isolation is the tight interdependence of micro-organisms with their environment and other microbial populations. On the other hand, several species cannot fully express their natural metabolic potential under strict laboratory conditions in pure culture. By mimicking natural environments and reducing the extant community diversity we have generated an intermediate system with lower complexity and yet allow unculturable species to grow in the consortium. Such a minimal ecosystem was achieved by serial diluting natural communities while preserving important ecosystem functions. Alternatively we can use synthetic communities that are created by combining a selected number of representatives of essential functional groups. I will discuss the current state of minimal and synthetic microbial mat communities and their potential use in facilitating novel bioactive compound discovery.

ORAL PRESENTATIONS - SESSION 3

COASTAL LAMINATED MICROBIAL ECOSYSTEMS ARE COMPOSED OF EUKARYA AND VIRUSES AS MUCH AS THEY ARE COMPOSED OF BACTERIA AND ARCHAEA

Silvia Cretoiu

NIOZ Royal Netherlands Institute for Sea Research, and Utrecht University, The Netherlands, silvia.cretoiu@nioz.nl

12:00 – 12:15, Thursday, 30 June 2016

Laminated microbial ecosystems occur in a wide range of extreme environments. They consist of non-random spatially distributed microorganisms that interact continuously. Examples of such microbial communities are the photosynthetic microbial mats that develop in intertidal areas. These mats are built by cyanobacteria that associate with a variety of other functional groups of microorganisms. Together they form a physical structure that stabilizes the sediment and promotes the colonization by plants. These microbial mats are like miniaturized Winogradsky columns and are characterized by vertical gradients of light, O_2, pH and H_2S. Their bacterial and archaeal representatives have been identified and their contribution to the nutrient cycling has been measured and are known. However, the role of viruses and Eukarya and the evolutionary forces that drive this microbial ecosystem are underexplored. Moreover, the molecular bases of the regulatory mechanisms that maintain the system as a whole and the interactions between different groups of microorganisms are largely unknown. Here, I conceive that the microbial mat composition and therefore its functionality is driven by two main factors: (1) a stable eukaryotic community and (2) an anti-viral immunity developed in the bacterial populations of the core community. The approach consists in investigating and interpreting the diversity and abundance of eukaryotic-protein markers and viral signatures as repetitive clustered regions together with their co-occurrence. Metagenome and network analysis of the microbial mats of the Dutch barrier island Schiermonnikoog confirmed the hypothesis and offered new insights in the function of microbial mats as living entities.

ORAL PRESENTATIONS - SESSION 3

MICROBIAL COMMUNITY STRUCTURE AND GENE EXPRESSION OVER A TIME COURSE OF 23 DAYS IN THE SOUTHWEST PACIFIC

Wolfgang R. Hess[a], Ulrike Pfreundt[a], France Van Wambeke[b], Mathieu Caffin[b], Dina Spungin[c], Ilana Berman-Frank[c], Sophie Bonnet[b]

a Genetics and Experimental Bioinformatics, Institute for Biology III, Faculty of Biology, University of Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany;

b Mediterranean Institute of Oceanography (MIO) – IRD/CNRS/Aix-Marseille University and IRD Nouméa, Nouméa, CEDEX, France;

c Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University Ramat Gan, Israel, wolfgang.hess@biologie.uni-freiburg.de

12:15 - 12:30, Thursday, 30 June 2016

N_2 fixation fuels ~50% of new primary production in the oligotrophic South Pacific Ocean. The VAHINE experiment was designed to track the fate of diazotroph derived nitrogen and carbon within a coastal lagoon ecosystem in a comprehensive way. For this, large-volume (~50 m^3) mesocosms were deployed in the New Caledonia lagoon and were intentionally fertilized with dissolved inorganic phosphorus to stimulate N_2 fixation. During days 15 to 23 of the VAHINE experiment, N_2 fixation rates increased dramatically, reaching >60 nmol N L^{-1} d^{-1}, which are among the highest rates reported for marine waters. This study examined the temporal dynamics of the prokaryotic community composition and gene expression together with the evolution of biogeochemical parameters for 23 consecutive days in one of these mesocosms (M1) and in the Nouméa lagoon. We observed clear successions within M1, some of which were not mirrored in the lagoon. In the lagoon, gene expression was dominated by the cyanobacterium *Synechococcus*, closely followed by alphaproteobacteria. In contrast, alphaproteobacteria dominated the gene expression in M1 until day 12, among them *Rhodobacteraceae*, rapidly gaining a high share in the metatranscriptome and peaking at day 4, followed by a burst in *Altermonadaceae*-related gene expression on days 8 and 10 and from *Idiomarinaceae* on day 10 in rapid succession. Thus, drastic dynamical changes in the microbial community composition and activity were triggered within the mesocosm already within the first 4 days, whereas the composition and activity of the lagoon ecosystem appeared more stable, although following similar temporal trends. We detected significant gene expression from *Chromerida*, suggesting these photoautotrophic alveolates were present in substantial numbers in the open water. Other groups contributing to the metatranscriptome were affiliated with marine *Euryarchaeota Candidatus* Thalassoarchaea and *Myoviridae* bacteriophages likely infecting *Synechococcus*, specifically inside M1. The high expression of genes encoding ammonium transporters and glutamine synthetase in many different taxa underscored the preference of most bacteria for this nitrogen source. The specific gene expression of diazotrophic cyanobacteria was attributed to *Trichodesmium* and *Richelia intracellularis* strains (diatom-diazotroph associations), both in the Nouméa lagoon and initially in M1. UCYN-A (*Candidatus* Atelocyanobacterium) transcripts were the third most abundant and declined both inside and outside after day 4, consistent with both 16S- and *nifH*-based analyses. Consistent with UCYN-C *nifH* tags increasing after day 14 in M1, transcripts related to the Epithemia turgida endosymbiont and *Cyanothece* ATCC 51142 increased from day 14 and maintained a higher share until the end of the experiment at day 23, suggesting these cyanobacteria were causing the observed high N2 fixation rates.

ORAL PRESENTATIONS

Session 4

Novel cultivation approaches of marine microbes

13:30 – 17:00, Thursday, 30 June 2016

Location: Bunsen Saal

Chair: Gaëtan Burgaud (Université de Brest, Technopôle de Brest Iroise, Plouzané, France)

Hitherto, only a small number of marine microorganisms have been brought into culture. It is of great importance to increase the number of marine microorganisms in culture because it is the only way to understand their nature, their function in the marine microbiome, and their properties, including potential pharmaceutical or biotechnological applications. Culturing the uncultured and the "unculturable" is MaCuMBA's motto as we seek to make progress after several decades of focus on cultivation-independent techniques. In this session we will discuss the state-of-the-art of isolation and culturing techniques and approaches, including co-cultivation of microorganisms and microbial consortia, use of environmental signals and cell-to-cell communication, and mimicking natural conditions.

ORAL PRESENTATIONS - SESSION 4

NEW APPROACHES FOR THE IMMOBILIZATION, CULTURE AND ISOLATION OF MARINE EXTREMOPHILES

Gwenaelle Le Blay

Institut Universitaire Européen de la Mer (IUEM) – UMR UBO-CNRS-IFREMER 6197, Laboratoire de Microbiologie des Environnements Extrêmes (LMEE), Place Nicolas Copernic, Université de Brest, Plouzané, France, gwenaelle.leblay@univ-brest.fr.

13:30 - 14:00, Thursday, 30 June 2016

Despite decades of efforts for microorganism culture and isolation, the vast majority of Bacteria and Archaea remains refractory to cultivation. For microbiologists, the current challenge is to leave the beaten track in order to develop innovative strategies that will increase the number of cultivated microorganisms. New strategies, based on cell immobilization and high-throughput techniques have been developed in the Laboratory of Microbiology of Extreme Environments for the cultivation and isolation of marine extremophiles. An immobilization protocol, using a heat-stable polymer matrix, has been developed for the culture of (hyper)thermophilic anaerobic marine communities. This protocol is based on a dispersion process in a two phase system composed of a polymer, and oil under agitation. The produced beads (1-2 mm in diameter) with immobilized hydrothermal microbial communities were then transferred in a gas-lift bioreactor maintained in continuous culture under anaerobic condition and constant dilution rate for several weeks. First, a synthetic community of 8 (hyper)thermophilic microorganisms was cultivated for 42 days at different temperatures (60°C & 85°C) in a rich culture medium. Cell quantification, organic acid concentrations and ATP monitoring showed that beads and liquid fractions (3 x 10^8 cells/ml) were highly colonized and that microorganisms were reactive to culture conditions. Several strains were maintained in the system and started to grow, even after a prolonged period of stress (pH and oxic stresses). A second microbial community, from a natural deep-sea hydrothermal vent chimney sample (Rainbow site) was immobilized and cultivated for 64 days in a low nutrient medium in different conditions. Total cell concentration remained low in the liquid fraction (6 x 10^5 cells/ml) all along the culture. Regular monitoring showed that ATP concentrations were generally higher in beads (up to 0.06 nmol/g) than in the liquid fractions (up to 0.02 nmol/ml). Tag sequencing and cloning sequencing showed that bacterial and archaeal species commonly found in hydrothermal vents were detected in the bioreactor, including uncultivated and difficult to grow taxa such as ε-Proteobacteria. The microbial diversity was higher in beads than in the liquid fractions although it decreased along the culture. In parallel, the Cocagne platform was designed to reach the high-throughput isolation of microorganisms by using a pipetting robot, allowing the handling of hundreds of miniaturized cultures in deep-well plates. Two approaches were tested, the dilution to extinction method developed by Giovannoni's team and the microencapsulation method developed by Zengler et al. In this approach, single cells are encapsulated in microdroplets of agarose that are incubated together in continuous culture. This technique was adapted for the microencapsulation of thermophilic microorganisms.

ORAL PRESENTATIONS - SESSION 4

ISOLATION OF SINGLE CELLS USING OPTCAL FORCES

Lynn Paterson, Anusha Keloth, Pierre-Henri Thiollier, Owen Anderson, Ajoy Kar, Gerard Markx

Institute of Biological Chemistry, Biophysics and Bioengineering,School of Engineering and Physical Sciences, Heriot-watt University, Edinburgh, EH14 4AS, UK, L.Paterson@hw.ac.uk.

14:00 - 14:15, Thursday, 30 June 2016

We report on the developments we have made to improve single cell isolation using optical forces. We have approached cell isolation in two ways. Firstly, using optical tweezers, where a single, tightly focused laser beam can trap microscopic particles in the beam focus. We have build an optical tweezers setup and written software to increase the number of cells collected and reduce the requirement for operator skill by making the procedure more automated, including image analysis and a mapping function. We have improved the throughput of cell isolation (from one cell per 30 minutes to one per minute) by automating the optical trapping, manipulation and delivery of single cells from a sample population into collection reservoirs. A second approach has been the design and fabrication of a three-dimensional microfluidic device. The microfluidic device is fabricated in fused silica using femtosecond laser inscription followed by selective chemical etching. The device is used to focus cells in a hydrodynamic flow. The optical scattering force from an inscribed waveguide is used to deflect single cells from the focused sample flow into the sheath flow, thus isolating single cells from the flowing sample population. The methods have advantages and drawbacks depending on cell size and throughput of experiment that is required by the user.

ORAL PRESENTATIONS - SESSION 4

THE "VIABLE BUT NOT CULTIVABLE" STATE FOLLOWS DIFFERENT PATHWAYS IN TWO VIBRIO SPECIES

Erica M. Prosdocimi[a,b], Elena Crotti[a], Zahraa Zeaiter[a], Stefania Arioli[a], Francesca Mapelli[a], Sara Borin[a]

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14:15 - 14: 30, Thursday, 30 June 2016

The Viable But Not Cultivable (VBNC) state is a physiological state in which bacterial cells are no longer cultivable on standard agar plates, which has therefore the potential to impair microbial isolation. *Vibrio* is one of the most studied marine bacterial genus known to enter into VBNC. From this state induced, for example, by starvation at 4°C, *Vibrio* cells can "resuscitate" if exposed to higher temperatures (30°C) prior to plating. Although several studies were performed on vibrios with regard to the VBNC transition, no direct comparison was tempted between species or strains. Hence, it is not clear if the results obtained with specific strains can be generalized. Moreover, the role of hydrogen peroxide was demonstrated in preventing the growth of VBNC cells, but it is still not clear if this is a universal mechanism or rather a specific pathway of *V. vulnificus*. Here, we monitored the loss of cultivability of four strains belonging to different *Vibrio* species (*V. harveyi, V. fortis, V. hepatarius* and *V. nereis*) during starvation in seawater at 4°C for 54 days. Cell morphological changes were described by means of flow cytometry. Two of them, *V. harveyi* and *V. fortis* were studied until the cells entered in VBNC. In both species during entrance in VBNC state we observed cell dwarfing, decrease in DNA quantity and a peculiar green fluorescence when the cells were stained with propidium iodide. These features were partially or totally reverted when cells resuscitated by incubation at 30°C. We, moreover, addressed the relationship between resuscitation and oxidative stress. Hydrogen peroxide as low as 0.007 mM prevented resuscitation in V. harveyi and a prolonged exposure to H_2O_2, far under the minimum concentration that inhibits growth, permanently damaged VBNC cells, which were not able to resuscitate anymore. Cultivability of VBNC cells was rescued, at least in part, by the addition of catalase in the growing medium to reduce cell oxidative stress. During the process of resuscitation both *V. harveyi* and *V. fortis* cells gradually increased their resistance to H_2O_2. The time and mode of the processes differed in the two tested strains, proving that the phenomena labeled as "VBNC" in different bacteria may be many, and not easily attributable to a unique pathway even in the context of a single genus.

ORAL PRESENTATIONS - SESSION 4

ISOLATION OF NOVEL MARINE HETEROTROPHIC BACTERIA USING LARGE-SCALE DILUTION-TO-EXTINCTION CULTURING

Christian Jeanthon[a,b], Delphine Duffieux[c,d], Klervi Crenn[a,b], Cendrella Lepleux[e], Delphine Serpin[a,b] and Jörg Overmann[e]

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e Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

14:30 - 14:45, Thursday, 30 June 2016

Cultivation of marine prokaryotes is challenging because a very low proportion (usually <0.1%) of the microbes readily form visible colonies on agar plates. This limitation has been partly overcome by novel cultivation techniques such as high-throughput culturing based on dilution-to-extinction[1]. High-throughput culturing was successful in particular in cultivating previously uncultured groups of marine bacteria, such as representatives of the SAR11 clade[2]. In the project MaCuMBa project, our objective was to isolate new pelagic free-living heterotrophic bacteria. Our isolation experiments evaluated different growth media using a dilution cultivation method in a 96-well microplate format. To simulate *in situ* conditions, low-nutrient heterotrophic media based on the local sea water were used. Micromolar concentrations of total organic carbon and inorganic nitrogen and phosphorous compounds were supplemented to favor the growth of oligotrophic microorganisms. The low-nutrient media were inoculated with pelagic samples collected offshore Roscoff, in the Bay of Naples, and in the Gulf of Aqaba. Our results showed that only a small proportion of growing cultures were able to form colonies on agar plates. Phylogenetic analyses of the 16S rRNA genes from 851 putative pure cultures demonstrated that numerous strains were obtained from abundant clades, such as OMG (e.g., SAR92 and OM60), marine *Roseobacter* clade, SAR116, OM182, and SAR11. We also developed an approach based on single-cell micromanipulation to isolate heterotrophic bacteria attached to marine microalgae. Our results showed that the culturable epibiotic microflora associated to diatoms is diverse and composed of numerous new taxa, some of which representing new genera. Our study showed that a large number of novel bacterial strains could be obtained by extensive cultivation applied to a few seawater samples.

1 Connon SA, Giovannoni SJ (2002) High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl. Environ. Microbiol. 68:3878-3885.

2 Rappe MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633.

ORAL PRESENTATIONS - SESSION 4

POLAR AND DEEP SEA MICROBIAL OBSERVATORIES

Halina E. Tegetmeyer, Katja Metfies, Ian Salter, Pier Luigi Buttigieg, Antje Boetius

Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27515 Bremerhaven, Germany and Max Planck Institute for Marine Microbiology, Bremen, Germany and Center for Biotechnology, Bielefeld University, Bielefeld, Germany, htegetme@cebitec.uni-bielefeld.de

14:45 - 15:00, Thursday, 30 June 2016

The range of environmental monitoring efforts targeting microbiomes in their assessments is steadily growing. In ecological systems, it is becoming increasingly clear that monitoring the phylogenetic diversity, functional potential, and activity of microbial communities is crucial in understanding element and nutrient cycling across environments. Indeed, microbial activity integrated over large spatial scales is of immense relevance for dynamics of the entire planet. Here, we summarise the use of microbiome data in the Frontiers in Arctic marine Monitoring (FRAM) programme for long-term marine monitoring (expedition.awi.de/fram). Annual samples of the marine water column and sediments at fixed sites along intersecting transects in the Fram Strait are being analysed with multi-omic approaches, complemented with data from a broad range of sensors and sampling devices assessing this system's biological and physico-chemical properties. Existing sequence-based data in this region have shown intriguing depth-related patterns in microbial diversity, across a range of resolutions associated with gradients of enzymatic activities and biogeochemical dynamics. Gradually, the knowledge generated by microbiome-focused investigations is being linked to the consequences of varying phytodetrital input and sea ice dynamics in this fragile system. FRAM will allow these signatures to be more fully explored, complemented with functionally oriented 'omics and key contextual data. This more holistic and temporally replicated approach is vital to better understand this region's rapidly changing ecology and better inform predictions and interventions in the understudied depths of the Arctic Ocean. A range of workflows and best practices are being developed to determine the most effective approaches to studying microbiomes in this long-term setting. Further, the outputs will be integrated into a larger system of ocean monitoring initiatives in the Atlantic through the AtlantOS project (www.atlantos-h2020.eu). While still in an early phase, we aim to further the role of microbiome analysis in marine monitoring and synchronize with other efforts across the biosphere.

ORAL PRESENTATIONS - SESSION 4

A NEW HYPOTHESIS ON THE NATURE OF MICROBIAL "UNCULTIVABILITY"

Slava S. Epstein

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Microbial "uncultivability" is arguably the oldest unresolved microbiological phenomenon. Most environmental species are yet to be cultivated, which hampers progress in microbial biology. The current explanations of the nature of the phenomenon have not bridged the gap between the large number of species in nature – and small in culture. This suggests these explanations may not be general enough. Here we propose a new hypothesis termed The Four Seasons of Microbial Life. The new model consists of three main postulates.

15:30 - 16:00, Thursday, 30 June 2016

1. Microbial species exit dormancy as a result of stochastic fluctuations in gene regulation. The low-frequency random events of awakening ("Spring") produce scout cells, who explore the environment and proliferate if the conditions are growth-permissive (or die otherwise). As in well-known bistability, this divides the isogenic population into two phenotypes, dormant and active. We postulated such stochastic transition in the "scout" hypothesis a few years ago, and have obtained empirical support in all species studied.
2. The successful scout forms a growing population ("Summer") that becomes progressively better adapted to the specific set of conditions into which it awoke, and the optimized pattern of gene expression is epigenetically inherited by the progeny. This leads to cells' differentiation, possibly to the point of being incapable of utilizing other conditions. This may be analogous to differentiation of pluripotent mammalian stem cells into a particular – yet isogenic - cell types. If such a differentiated microbial population is transferred to a different environment, e.g. Petri dish, it may not be able to grow – unless de-differentiation takes place. This proposal finds support in the phenomenon of diauxic shift discovered by J.Monod in 1941: a population of *E. coli* that utilized glucose as the single C source is incapable of utilizing lactose. In our terms, *E. coli* differentiates to the glucose environment and becomes "uncultivable" in the lactose habitat.
3. Once the resources have been exhausted ("Fall"), the population goes dormant, completing the cycle. We propose that inactivity, apart from survival function, de-programs cells ("Winter") because they "forget" the former gene expression pattern. If so, dormancy returns the cells to their pluripotent state, from which they can wake up and proliferate under a number of different scenarios. If the range of such growth-permissive scenarios includes artificial media, the population will appear cultivable on such media, whereas the phenotype differentiated in the environment will not.

We argue that the Four Seasons of Microbial Life hypothesis is consistent with all empirical observations related to the Great Plate Count Anomaly. It helps explaining the nature of VBNCs, persister cells, and other microbiological phenomena. It also suggests a simple practical way to cultivate "uncultivable" species.

ORAL PRESENTATIONS - SESSION 4

QUORUM SENSE AND QUENCHABILITY IN THE OCEANS

F. Jerry Reen[a], José A. Gutiérrez-Barranquero[a], Claire Adams and Fergal O'Gara[a,b]

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16:00 - 16:15, Thursday, 30 June 2016

Language diversity has proven a major barrier to human relations over centuries and unlocking the subtle nuances of colloquial speech has provided significant advantages to the enlightened few. Much in the same way, understanding the capacity of microbes to translate diverse languages and adapt to the presence of other organisms, is providing an important new direction to the study of microbial systems. Classified under the generalised term of quorum (threshold) sensing, the best characterised of the microbial languages is the N-acyl homoserine lactone (AHL) quorum sensing system, which is widely distributed among Gram negative bacteria. Often described as a form of cellular warfare, the secretion of signals has the capacity to facilitate the growth of some organisms while antagonising the growth of others. Microbes appear equally equipped to quench these conversations, with mimic and enzyme based systems employed to silence communication in response to external cues. Deciphering the underlying mechanisms through which these sensing and quenching systems operate provides us with an opportunity to greatly enhance the isolation of microbial diversity from the marine environment, thus unlocking the silent potential of this rich ecosystem. However, identification and characterisation of these systems is in its infancy and relatively little is known about the abundance, nature and diversity of quorum sensing systems in the marine environment. Through the MaCuMBA programme, we have uncovered a diverse array of communication signals and their quenching counterparts in isolates from marine sponges harvested off the coast of Ireland. The nature of these novel signals was elucidated using a combination of genomic, chromatographic and electrovoltammetric technologies. Supplementing growth media with supernatants from QS positive organisms resulted in the consistent recovery of a greater diversity of morphologies when compared to controls. A filter-based co-culture system for extended exposure to QS signals and QQ mimics also revealed a significant increase in diversity of recoverable isolates. Taxonomic profiling has enabled us to classify at species level, with phenotypic assays providing important new knowledge on the impact of QS and QQ on marine microbial behaviour. In addition, the collection of signalling and quenching isolates has delivered a suite of extracts that interfere with a key marker for microbial pathogenesis and persistence, namely biofilm formation. Microbial aggregation in the form of matrix embedded biofilm structures is a key factor in the decline of effective antibiotics, while biofouling of marine structures represents a significant economic burden. Marine small molecule mimics that block the formation of biofilms have the potential to re-establish the effectiveness of conventional antibiotics, and may help to prevent the onset of the 'post-antibiotic era'.

ORAL PRESENTATIONS - SESSION 4

ISOLATING MARINE BACTERIA: A CULTIVATION GUIDE

Cendrella Lepleux, Johannes Sikorski, Amber H. Scholz and Jörg Overmann

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16:15 - 16:30, Thursday, 30 June 2016

The composition of marine bacterioplankton communities can be accessed through culture-independent approaches but there have been only limited opportunities to determine their metabolic potential mostly due to their low cultivation success of frequently <0.1%. At the same time, the high fraction of physiologically active bacteria in natural marine samples indicate that these bacteria are capable of dividing and that previous cultivation methods are not optimally suited to meet the growth requirements of these microorganisms. With little more than 10,000 isolated species, we are still far away from the 10^7 to 10^9 bacterial species estimated in total. Among the currently known and cultivated bacterial groups some are underrepresented such as the class of *Flavobacteria*, numerous *Alphaproteobacteria* genera as well as the *Chloroflexi* phylum. This raises the question how new bacteria from specific groups/phyla can be cultivated. Using three different sets of marine samples from the Baltic sea, the Channel sea and the Atlantic ocean and employing two principally different cultivation approaches and 5 growth media, we analyzed the positive or negative selection of the cultivation method on bacterial group/phyla. A high-throughput cultivation method was applied with 5 media differing in their carbon complexity (traces of carbon substrates, soil extract, soil extract treated with UV, insoluble or soluble humic analogs, polymer mix). In parallel, the samples were also used to test enrichments on inert surfaces using one medium (traces of carbon substrates). Among all the media tested, the one with traces of carbon substrates (1:10HD ; glucose, peptone and yeast extract) showed the highest cultivation success while the lowest value was found with the medium containing polymers (cellulose, chitin, pectin, xylan and curdlan). The isolation results also showed a higher bacterial diversity retrieved with 1:10 HD medium than with the other media where a more specific bacterial community was found. A different bacterial diversity was recovered by the two cultivation methods, with the *Alphaproteobacteria* and the *Gammaproteobacteria* selectively isolated through the surface experiment. Across all the experiments 702 strains were analyzed, of which 23 strains had a 16S rRNA identity <96% compared to existing type strains and hence represent novel bacterial genera, while 113 strains had a similarity <98% and represent novel bacterial species. The analysis of these results will allow us to suggest “cultivation best practices” according with a specific bacterial group or phylum.

ORAL PRESENTATIONS - SESSION 4

EFFECT OF NUTRIENTS AND GELLING AGENT ON CULTURABILITY OF MARINE BACTERIA

Anita M. Rygaard, Eva C. Sonnenschein, Mikkel Bentzon-Tilla, Lone Gram, Mariane S. Thogersen

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16:30 - 16:45, Thursday, 30 June 2016

Bacterial isolates obtained using traditional cultivation methods represent only about 1% of the total microbial diversity. Culturability is crucial for biodiscovery of novel compounds such as industrial enzymes and antibiotics. While metagenomic sequencing approaches have shown that the marine environment harbors a great resource of unknown microorganisms, their potential for production of novel bioactive metabolites remains mostly uncovered due to lack of cultivation. To make these bacteria accessible for bioprospecting through cultivation, we investigated the effect of gelling agent on the culturability of marine bacteria sampled from a Danish harbor using a high nutrient medium (Marine Broth 2216 (MB)) versus a low nutrient medium (filtered seawater) – both solidified with either agar or gellan gum. We also tested the effect of incubation temperature, time, and addition of a mix of naturally occurring quorum sensing molecules (AHLs). Culturability (colony forming units (CFU)/ml) was compared to total cell count (5.8 x 10^6 cells/ml) as determined by SYBR Gold staining. CFU was highest on MB gellan gum media with a cultivation success of up to 3.4% at 10°C and 6.4% at 25°C. For the corresponding MB agar medium, culturability was 0.5% and 2.1% at 10°C and 25°C, respectively. For the low-nutrient media, culturability was much lower; ranging from 0.004% for agar at 25°C to 0.2% for gellan gum at both 10 and 25°C. Prolonged incubation times from 5 to 10 weeks at 10°C and 2 to 5 weeks at 25°C only increased number of CFUs significantly on MB gellan gum and MB agar at 25°C. Addition of two different concentrations of mixed AHLs (0.5 µM and 50 µM) did not increase the culturability on any of the agar-solidified media (gellan gum was not tested). To assess the effect of growth conditions on the microbial diversity, amplicon sequencing of the V4 region of the 16S rRNA gene was performed from colony material and from biomass obtained from direct filtration of seawater. All samples were dominated by α-, β-, and γ-Proteobacteria ranging from a 74% of total diversity on MB gellan gum to 99% on MB agar. The filtered seawater contained a total of 50-82% of α-, β-, and γ-Proteobacteria. MB gellan gum supported the growth of actinobacteria (≤ 15% of total diversity) compared to the remaining media (≤ 1%). The seawater contained a higher diversity including δ-Proteobacteria (≤ 3%), Sphingobacteria (≤ 5%), and several “unclassified” bacterial classes (≤ 5%) compared to the substrates. Only the MB gellan gum medium supported substantial growth of unclassified classes (≤ 2%), and measures of β-diversity showed that the MB gellan gum medium clustered with the filtered seawater, suggesting gellan gum to be able to support growth of the microbial communities of seawater better than agar. Concluding, we found that culturability in terms of CFUs and bacterial diversity could be improved substantially using gellan gum instead of the traditional agar, making it a promising solution for future bioprospecting.

ORAL PRESENTATIONS - SESSION 4

ORGANISATION OF A NOVEL FUNGUS TRIGGERED BY A MICROFABRICATED ENVIRONMENT

Colin Ingham and Laura Caton

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16:45 - 17:00, Thursday, 30 June 2016

Culture chips are arrays of miniaturized wells (20 to 200 µm diameter, 4000 to 250,000 wells) with a porous ceramic base. These can be used to grow and screen marine microorganisms. A chip-based screening was undertaken for microorganisms derived from marine sponges; a colony with an unusual morphology was observed. Identification using 18S-related sequences suggested that the isolate was fungal, a new species of true *Acremonium*. Electron microscopy and imaging of growth suggested that culture in microwells wells promoted the formation of hydrated, dye-incorporating vegetative structures composed of parallel bundles of mycelia with a topological developmental trigger.

In order to explore how physical and topological factors triggered development, new chips were constructed. These chips varied parameters such as well size, well spacing, wall height, chemical functionalization, surface roughness, porosity and hydrophobicity. Cultivation on these chips was used to understand how the physical environment triggers long term changes in fungal morphology, including a screen of other fungi.

More broadly, microfabricated culture devices have applications in marine cultivation and ecology.

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Stefanie P. Glaeser
Department of Applied Microbiology, Justus Liebig University Giessen, Germany

2. ***EMBRIC – DISCOVERY AND EXPLOITATION OF NOVEL MARINE PRODUCTS AND BIOMOLECULES: SECONDARY METABOLITE PIPELINE***
Torsten Meiners
FMP Leibniz-Institute of Molecular Pharmacology, Berlin, Germany

3. ***EXPLORATION OF THE MICROBIOME IN THE ICELANDIC OCEANS BY CULTIVATION AND NEXT GENERATION SEQUENCING (NGS)***
Alexandra Klonowski
Matís ohf./Food Safety, Environment and Genetics, Vínlandsleið 12, 113 Reykjavík, Iceland

4. ***EMBRIC PROTEIN PLATFORM (NO ABSTRACT)***
Carlos Lopes
CCMAR, Faro, Portugal

5. ***CULTURING AND MUTAGENESIS OF MARINE SYNECHOCOCCUS***
Blanca Perez Sepulveda
School of Life Sciences, University of Warwick, UK

6. ***EU-OPRENSCREEN (NO ABSTRACT)***
Philip Gribbon
FMP, Berlin, Germany

7. ***MICROMBT: MICROBIAL COMMUNITIES AND MOVING BED TECHNOLOGY AS TOOLS FOR CONVERSION OF MARINE BIOMASS***
Concetta De Santi
Department of Chemistry, UiT-The Arctic University of Norway

8. ***SPATIO TEMPORAL DISTRIBUTION OF BACTERIA AND PICOPLANKTON IN THE SOUTHERN BENGUELA UPWELLING REGION***
Emma Rocke
University of Cape Town, Department of Biological Sciences, South Africa

9. ***MACHINED OPTICAL FIBRES FOR ON-CHIP OPTICAL TWEEZING***
Georgia Anastasiadi
Institute of Photonics and Quantum Science, School of Engineering and Physical Sciences, Heriot-watt University, Edinburgh, UK

10. ***DE.STAIR: A NEW INITIATIVE FOR THE STRUCTURED ANALYSIS AND INTEGRATION OF RNA-SEQ EXPERIMENTS***
Steffen Christian Lott
Genetics and Experimental Bioinformatics, Institute for Biology III, Faculty of Biology, University of Freiburg, Germany

11. ***PHOTOREDUCTION OF SILVER NANOPARTICLES IN GLASS TO CREATE SERS MICROFLUIDICS FOR ENHANCED MOLECULAR SENSING***
Mark Mackenzie
Institute of Photonics and Quantum Sciences, School of Engineering and Physical Sciences, Heriot Watt University, Edinburgh, UK

12. ***RHIZOBACTERIA OF THE SEAGRASS ZOSTERA MARINA HAVE A WIDE ARRAY OF SULFUR GENES BENEFICIAL FOR THEIR HOST***
Catarina Cúcio
Microbial Systems Ecology, University of Amsterdam, The Netherlands

13. ***TAXONOMIC AND TRANSCRIPTIONAL DYNAMICS OF THE MICROBIAL COMMUNITY DURING A TRICHODESMIUM BLOOM***
Shengwei Hou
Genetics and Experimental Bioinformatics, University of Freiburg, Germany

POSTER PRESENTATIONS - INDEX

14. ***OPENING UP THE BLACK BOX OF MARINE PHOTOTROPH-HETEROTROPH INTERACTIONS***
Alicja Dabrowska
School of Life Sciences, University of Warwick, UK

15. ***A NOVEL STRATEGY FOR EXPLOITATION OF HOST RNASE E ACTIVITY BY A MARINE CYANOPHAGE***
Claudia Steglich
Faculty of Biology, University of Freiburg, Germany

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Jens Harder
Department of Microbiology, Max Planck-Institute for Marine for Marine Microbiology, Bremen, Germany

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Manuela Schüngel
DMSZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

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Scripps Institution of Oceanography, La Jolla, USA

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EMBRC-ERIC Headquarters, Paris, France

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POSTER PRESENTATIONS - 1

CULTIVATION OF BACTERIAL CONSORTIA FROM EUKARYOTIC MARINE HOSTS

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Marine coral and sponges are holobionts of a eukaryotic host and diverse host-associated microbial communities. These communities interact with the host and among each other, and play a key role for the health status of the host. The interaction is mainly regulated by the production of different secondary metabolites produced by the various microbes. Secondary metabolites drive the microbial community composition by microbe-microbe interactions and protect both the host and the host-specific microbial consortia from pathogens. In our point of view, the communication of the different partners in those microbial consortia play a key role in the cultivability of so far non-culturable or not-yet cultured microorganisms. Through a combination of different co-cultivation strategies, we are aiming at a specific enrichment and isolation of stable interacting microbial consortia, to understand the role of the interaction and the cultivability of those microbes. Here we present first results of dilution to extinction cultivation experiments from corals and sponges of the CEMarine marine aquaculture system in Giessen, Germany.

POSTER PRESENTATIONS - 2

EMBRIC - DISCOVERY AND EXPLOITATION OF NOVEL MARINE PRODUCTS AND BIOMOLECULES: SECONDARY METABOLITE PIPELINE

Torsten Meiners, Philip Brennecke, Mark Brönstrup, Mirjam Czjzek, Michael Hensler, Huanting Liu, James H. Naismith, Deborah Power, David Smith, Philip Gribbon

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Marine metagenomics is revealing the extraordinary diversity, complexity and variability of microbial communities in the oceans, emphasising the potential of prokaryotes and micro-eukaryotes as banks of novel chemical substances. Genome scanning shows that only a small fraction of the compounds that marine microbes are genetically capable of producing have so far been isolated. Natural product discovery pathways involve distinct technologies and expertise in areas such as extraction, fractionation, compound isolation and purification, determination of biological activities, as well as molecular and structural characterization of marine primary or secondary metabolites. This expertise exists in different European research infrastructures, but is fragmented and not always clearly accessible to biotechnology users. In this context, the EMBRIC secondary metabolite pipeline will offer access to culture collections of marine microbes, to explore them for novel lead compounds or bioactive products for use in health care and nutrition for humans, as well as for use as immune-modulators in agriculture and aquaculture. The EMBRIC secondary metabolite pipeline is currently assembling existing capacities into consolidated and coherent service-oriented workflows with organized and catalogued services and facilities for:

- Enabling basic bioprofiling of marine extracts to assess the presence of interesting antibacterial, antifungal, antiparasitic, cytotoxic and anti-proliferative activities against relevant pathogens and cell lines;
- Access to Standard Operating Procedures and best practices for downstream processing of biological resources including extract preparation, fractionation, advanced analytics such as high resolution mass spectroscopy and nuclear magnetic resonance spectroscopy for compound identification, preparative isolation, and chemical structure elucidation;
- Access to expert databases and tools for rapid dereplication of analytical signatures of compounds to chemical structures in order to identify new chemical entities and discriminate from already known molecules;
- Offering user assistance in the chemical hit optimization process;
- Ensuring quality control, maintenance of sufficient supply, and distribution of pure isolated marine secondary metabolites for seamless incorporation into open-source European collections;
- Supporting the development of novel production processes, including the heterologous expression of cloned or chemically-synthesized metabolite synthesis gene clusters in order to mitigate unreliable supply of biomass often encountered with marine organisms.

POSTER PRESENTATIONS - 3

EXPLORATION OF THE MICROBIOME IN THE ICELANDIC OCEANS BY CULTIVATION AND NEXT GENERATION SEQUENCING (NGS)

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Despite their importance for oceanic primary production and marine food webs, little is known about the diversity of microorganisms – bacteria, archaea and eukaryotic protista – in Icelandic marine waters. Research cruises have therefore been initiated in 2010 and were conducted during May in each year since then. During the cruises, water samples are taken around all of Iceland and at different depths for the cultivation of microorganisms and "non-cultivation" analyses, like Next Generation Sequencing (NGS) and Flow Cytometry. In addition, accompanying oceanographic data are collected with the goal to better understand the marine microbial diversity and its changes under changing environmental conditions. During the Macumba project, around 100 of those Icelandic ocean samples have been enriched on different solid and liquid media, mimicking the natural composition of sea water, and at different temperature regimes. Results have shown that the largest diversity was obtained at 4°C and it was essential to use solid medium instead of a liquid culture to increase the diversity. Around 1.200 strains of bacteria have been isolated and deposited to the Icelandic Strain Collection And Records (ISCAR). Characterization of strains comprises of MALDI-ToF analysis, sequencing of the 16S rRNA gene – and in some cases of the whole genome – and a morphological description. More than 200 of the strains were identified based on their 16S rRNA gene sequence and a following comparison with sequence databases. Isolated strains belonged for example to the genera *Alteromonas, Colwellia, Dietza, Halomonas, Marinobacter, Pseudoalteromonas, Rhodococcus* and *Sulfitobacter*. However, most strains (62%) showed only a higher identity to database entries from uncultivated and therefore unclassified and uncharacterized samples that were derived from metagenomic sequencing of whole environmental samples, or they were completely unknown. These putative novel bacterial strains or species are prioritized for further characterization and whole genome sequencing.

POSTER PRESENTATIONS - 5

CULTURING AND MUTAGENESIS OF MARINE SYNECHOCOCCUS

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Phytoplankton is responsible for ca. 50% of global primary production and encompasses a wide range of microorganisms of which *Synechococcus* is a key marine genus. Despite being ubiquitous in the ocean and playing a major role in the marine carbon cycle, the isolation and characterization of some *Synechococcus* clades, known only from cultivation-independent approaches, still presents a real challenge. Here, we present a new approach for isolation of marine *Synechococcus*, which encompasses the use of heterotrophic bacteria. These heterotrophic bacterial 'helpers' improve colony growth and plating efficiency of the co-occurring *Synechococcus*. Moreover, we have also specifically engineered a *Roseobacter pomeroyi* strain, to facilitate a much-improved targeted mutagenesis protocol for use in marine Synechococcus.

POSTER PRESENTATIONS - 7

MICROMBT: MICROBIAL COMMUNITIES AND MOVING BED TECHNOLOGY AS TOOLS FOR CONVERSION OF MARINE BIOMASS

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There is an urgent need to develop new and green technologies for use of rest-raw material from the fish industry. It is estimated that of the 7 million tons of fish caught by North Atlantic nations, at least 3.5 million tons ends up as rest material. Inspired by the RAS (Recirculating Aquaculture system) technology, the MicroMBT project aims at using Moving Bed Technology (MBT) to optimize microbial communities for conversion of cheap biomasses into new products and increase the value of marine biomasses. Optimized microbial communities (i.e., the biocatalyst) will originate from marine bacterial isolates collected from the Arctic region. Each isolate has been screened for specific activities (e.g., cellulases, lipases, proteases), genome sequenced and analyzed for their secondary metabolites production potential using a computer-based approach (e.g., the Anti-smash software). Based on the microbial genetic and metabolic potentials, the bacterial communities will be established as biofilm on the surface of plastic beads and used as microfactories in bioreactors, in which enzymatically processed fish rest raw materials (peptide hydrolysates) will be used as the input medium. In micro- and lab-scale bioreactors, the reactor conditions including, temperature, pH, oxygenation, bio-beads size/type and combinations of microbes will be monitored and optimized for efficient conversion of biomaterials. Ultimately, the scalability of the biorefinery process will be tested by performing a pilot-scale application under industrial process conditions. Spent medium will be collected and subjected to untargeted and targeted analytic analyses to produce global metabolome profiles and measurements of potential bioproducts of commercial interest, respectively.

Keywords
Moving Bed Technology, Recirculating Aquaculture System, metagenomics, metabolomics, microbial communities

POSTER PRESENTATIONS - 8

SPATIO TEMPORAL DISTRIBUTION OF BACTERIA AND PICOPLANKTON IN THE SOUTHERN BENGELA UPWELLING REGION

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Picoplankton (0.2-2µm in diameter) are responsible for fixing up to 50% of CO_2 and are the most abundant photosynthetic organisms in the ocean. This size fraction of phytoplankton however has been poorly studied, and to date there exists virtually no picoplankton data from the productive Southern Benguela upwelling. The Southern Benguela, situated on the West coast of South Africa is characterized by a pulsed, seasonal wind driven upwelling at various coastal points accompanied by warm Agulhas water offshore. High primary productivity enriches the coast, creating nursery grounds for several fish species. Low oxygen events inshore affect these resources. Through seasonal cruises, both flow cytometry and DNA surface samples were sampled and analyzed. Preliminary results indicate a dinoflagellate (*Ceratium*) bloom (in it's final stages) in May in St Helena Bay (Figure 5), accompanied by *Prochlorococcus* and *Synechococcus* presence up to 1.4 x 10^4 cells/ml (Figure 2 & 3). As expected, due to depleted nutrient conditions post bloom, Prochlorococcus, whom do better in low nutrient conditions, were higher in numbers at this time compared to *Synechococcus*. The September (winter) sample analysis revealed very few *Prochlorococcus*, however a significant amount of *Synechococcus* was present in the northern stations at this time. *Synechococcus* are known to have an affinity to temperate mesotrophic waters, and so their presence here is in line with previous research focusing on *Synechococcus*. Furthermore, upon flow cytometry analysis of *Synechococcus* populations specifically, it was noted that the phycoerythrin content shifted depending on depth (ie: higher content at Fmax versus surface waters). This could be an adaptation mechanism in response to shifting light conditions, as well as genetic differences, or different sub species occupying specific ecological niches. 16S rDNA pyrosequencing results also revealed separate sub clades of *Synechococcus* for different water depths. These results indicate that *Synechococcus*, known to be one of the most important components of prokaryotic autotrophic picoplankton, play a significant role in the Southern Benguela upwelling region. As well as *Synechococcus*, 16S rDNA sequence data revealed a significantly higher prokaryotic diversity in OMZ samples compared to surface samples.

POSTER PRESENTATIONS - 9

MACHINED OPTICAL FIBRES FOR ON-CHIP OPTICAL TWEEZING

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Optical Tweezers is a technique for manipulating micron scale particles using the momentum of light caused by the radiation pressure exerted from a laser beam. It has extensively developed over the last 30 years, and has seen widespread applications. This technique is non-invasive so that it can be applied to biological cells without damaging them, as well as it can enable a variety of single cell experiments or cell-cell communication experiments. To date, optical tweezers tend to be based on a high numerical aperture microscope objective to deliver the tweezing light and image the sample. This introduces restrictions upon the optical microscope. We aim to overcome these limitations by developing a different approach, based on optical fibre delivery of the tweezing light. This approach is based on micromachining the optical fibres and the fabrication of bespoke microchannels on microscope slides in order to construct small, portable devices capable of manipulating cells under any microscope. We have demonstrated two opposed optical fibres that create two counter-propagating laser beams. A micro-particle is trapped at the point where the two scattering forces exerted from the two beams are equal. The fibres are held in microchannels, which have been fabricated in glass microscope slides, using a picosecond-laser welding process. To create a stronger, more stable fibre trap we are micromachining mirrors on the end of optical fibres so that the laser light can be directed from multiple cores to form an optical trap. The microfabrication of the fibre ends has been demonstrated using Focused Ion Beam etching (FIB). This technique etches and removes material with a beam spot size of 1 Angstrom. It uses a liquid Gallium ion source, which is connected to a tungsten needle. Once the Gallium heats, it wets and runs to the end of the 2nm diameter tip. Electrostatic lenses accelerate the Gallium ions and focus them onto the sample surface where the beam removes a small amount of material. We investigate the FIB etching parameters necessary to achieve optical trapping from micromachined fibres and have explored a variety of fibre configurations, such as multiple single-core fibres and multi-core fibres. The use of a single optical fibre to stably manipulate a cell in three dimensions, independently from the microscope objective of, for example a Raman microscope or super-resolution microscopes such as Total Internal Reflection Fluorescence microscope (TIRF) will enable a range of new experiments. These, typically facility-owned microscopes, cannot be readily adapted to send a tweezing beam through their microscope objective lenses, so fibre tweezers offer an alternative.

POSTER PRESENTATIONS - 10

DE.STAIR: A NEW INITIATIVE FOR THE STRUCTURED ANALYSIS AND INTEGRATION OF RNA-SEQ EXPERIMENTS

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Transcriptomic and metatranscriptomic (RNA-Seq) approaches provide comprehensive insight into gene expression at the genome-wide or biome-wide level at single-nucleotide resolution. However, the variety of protocols, experimental study designs and the characteristic properties of the organisms under investigation greatly affect downstream and comparative analyses. Therefore, the in-depth analysis of RNA-Seq experiments can constitute substantial challenges to the experimentalist. The de.STAIR project is currently being established within the frame of the German Network for Bioinformatics Infrastructure (https://www.denbi.de/) and aims at providing tools and workflows for the overall integration of transcriptomic data towards additional regulative, predictive and annotation capabilities. One element within de.STAIR is the characterization of bacterial small RNAs (sRNAs). Whereas hundreds-to-thousands sRNAs are identified in individual transcriptomic analyses, their large-scale functional characterization is currently impossible and as a consequence these important regulators of gene expression are frequently neglected. Within de.STAIRS we want to tackle this problem biocomputationally and present a tool that reliably finds homologous sRNAs in large databases. GLASSgo (GLobal Automated sRNA Search go) combines iterative BLASTn searches, pairwise identity filtering, and structure based clustering in an automated prediction pipeline. In order to test the performance of GLASSgo, we carried out a benchmark with all sRNA families from the latest Rfam[1] release. The results show that GLASSgo reliably predicts related sRNAs for a given query. Besides the basic functionality, GLASSgo will be useful for the research community in various ways. For example, sRNA target prediction is a common task in the characterization of an sRNA, and this it at best performed by taking into account evolutionary conservation. A widely used tool for this is CopraRNA[2, 3], which takes as input a set of conserved sRNAs and finds conserved target mRNAs. Additionally, de.STAIR will offer in the near future the comprehensive analysis of RNA-Seq experiments as a service.

References

1 E. P. Nawrocki, et al., Nucleic Acids Research 43, D130 (2015).
2 P. R. Wright, et al., Proc Natl Acad Sci 110, E3487 (2013).
3 P. R. Wright, et al., Nucleic Acids Research 42, W119 (2014).

POSTER PRESENTATIONS - 11

PHOTOREDUCTION OF SILVER NANOPARTICLES IN GLASS TO CREATE SERS MICROFLUIDICS FOR ENHANCED MOLECULAR SENSING

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A Raman signal is a molecular fingerprint, generated when light elastically scatters from a sample. Raman spectroscopy has applications in detecting and phenotyping bacteria. The integration of Surface Enhanced Raman Spectroscopy (SERS) measurement techniques with microfluidics has the potential to produce compact, high throughput and low cost molecular sensing devices. We have fabricated microfluidic devices by focusing a 1047 nm femtosecond laser into a fused silica substrate where the light is absorbed through multi photon processes to modify the material. Modified regions were removed through hydrofluoric acid etching. This is an inherently 3D modification technique for fabricating microfluidics allowing us to create complex device shapes. Using the same laser we selectively patterned silver nanoparticle SERS substrates into the device by photoreduction. Initial experiments to detect the Raman signal from Rhodamine 6G dye have shown twenty times enhancement of the Raman signal from the silver nano-patterned surface compared to unmodified fused silica. We have investigated how the experimental parameters used for photoreduction impact the structure of the patterned silver nanoparticles and how the structures enhance the Raman signal. Combining SERS and microfluidics holds promise to increase the Raman signal, reduce the analyte used and increase the throughput of the experiment.

POSTER PRESENTATIONS - 12

RHIZOBACTERIA OF THE SEAGRASS ZOSTERA MARINA HAVE A WIDE ARRAY OF SULFUR GENES BENEFICIAL FOR THEIR HOST

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Seagrass meadows are marine habitats that cover up to 600,000 km^2 of the world's coastal areas. The most widespread seagrass species, *Zostera marina*, is an important ecosystem engineer in these areas, and is one of the species responsible for the maintenance of the remarkable productivity registered in these ecosystems. However, seagrass meadows are in decline mostly due to anthropogenic causes, such as increased eutrophication and water turbidity, which enhance the depletion of oxygen and boost anaerobic metabolism of bacterial communities in the surroundings of seagrass roots the rhizosphere. The high levels of organic matter naturally present in sediments of seagrass meadows fuel the activity of sulfate-reducing bacteria under anaerobic conditions, resulting in the release of sulfide, which is toxic for the seagrasses. Using Illumina HiSeq shotgun sequencing, we analysed the metagenome of the rhizosphere of *Z. marina*. Analysis of 16S rRNA reads revealed a dominance of members of the families *Chromatiaceae* and *Desulfobacteraceae* from the *Gamma-* and *Deltaproteobacteria*, respectively. Preliminary results based on the number of gene copies and respective coverage of marker genes involved in both the oxidative and reductive sulfur cycle, indicate that the adenosine 5'-phosphosulfate (APS) pathway has the highest genetic potential in dissimilatory sulfur metabolism, followed by the flavocytochrome *c* sulfide dehydrogenase and the sulfide-quinone reductase pathway. Phylogenetic analysis of the reductive dissimilatory sulfite reductase (*dsr*AB) revealed that this gene is mainly represented by members of the family *Desulfobacteraceae*. Furthermore, gene copies of the sulfur oxidation genes *sox*B and reverse-operating *dsr*AB clustered preferentially with symbiotic sulfur-oxidizing bacteria. These results highlight the importance of sulfur bacteria in the rhizosphere of *Z. marina*, and their possible role in the detoxification of sulfide, which is beneficial for the seagrass.

POSTER PRESENTATIONS - 13

TAXONOMIC AND TRANSCRIPTIONAL DYNAMICS OF THE MICROBIAL COMMUNITY DURING A TRICHODESMIUM BLOOM

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The complex metabolic and regulatory microbial interactions occurring between diverse species within algal and cyanobacterial blooms have been investigated for decades. Characteristic taxa following the demise of such blooms were observed repeatedly in the natural environment. However, the puzzle of interactions and functional dynamics among these bacteria is not fully understood thus far. Here we have applied a combined metagenomic and metatranscriptomic approach to study the microbial community composition and transcriptional dynamics within a *Trichodesmium* bloom in the New Caledonia lagoon in the Southwest Pacific. *Trichodesmium* is a nitrogen-fixing cyanobacterium of substantial ecological importance. Metagenomic decomposition analysis showed that *Oscillatoriophycideae* (mainly *Trichodesmium*) and *Alteromonadaceae* dominated the bloom, accounting for 39.5% or 35.2% of all metagenomic reads, respectively. The third and fourth-most abundant families were *Pseudoalteromonadaceae* and *Myoviridae*, which made up 2.7% and 2.3% of the community DNA pool. The other families taking up more than 0.5% of the community were *Vibrionaceae* (1.7%), *Saprospiraceae* (1.2%), *Flavobacteriaceae* (1.2%), *Enterobacteriaceae* (0.8%), *Lactobacillaceae* (0.6%) and *Rhodobacteraceae* (0.5%). During the demise, the metatranscriptional activity of *Oscillatoriophycideae* dropped drastically, from initially 63.3% of all reads to 7.3% in the first 4h, and to 4.5% in the following 4h. In contrast, the share of *Alteromonadaceae*-related reads increased from 0.4% to 57.5% in the first 4h, and increased further to 67.5% in the next 4h. Following the pattern of *Alteromonadaceae, Pseudoalteromonadaceae* increased from 0.21% to 2% in the first 4h, and to 4.1% in the second 4h. In contrast, *Saprospiraceae*–related reads decreased from 4.5% to 0.6% and 0.08% in the measured time periods, indicating a similar dynamic pattern as the *Oscillatoriophycideae.* Interestingly, *Myoviridae* increased from the share of 0.09% to 0.46% during the first 4h, then boosted to 2.1% in the second 4h, correlated with the abundancy curve of *Alteromonadaceae*. A major part of these reads belonged to a type of giant bacteriophage, not observed before in the marine environment Deep metatranscriptomics analysis showed that *Alteromonadaceae* up-regulated the expression of genes encoding proteins for outer membrane assembly (BamD), colicin uptake (TolB), cyanophycin degradation (cyanophycinase), polysaccharide hydrolysis (pullulanase), and hydrolases (e.g., cysteine protease YraA). Down-regulated genes were related to iron-storage (bacterioferritin), drug-resistance (Multidrug resistance protein MdtL), glycolysis and gluconeogenesis (fructose-bisphosphate aldolase class 1), nitrogen fixation (nitrogenase molybdenum-iron protein alpha chain, hydrogenase/urease nickel incorporation protein HypA). Our data illuminate the rapid succession of taxa during a rapidly deteriorating *Trichodesmium* bloom and highlight key transcriptional activities within the involved species.

POSTER PRESENTATIONS - 14

OPENING UP THE BLACK BOX OF MARINE PHOTOTROPH-HETEROTROPH INTERACTIONS

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Although marine microorganisms drive the major biogeochemical cycles in marine ecosystems, there is a dearth of information on interactions between phototrophic and heterotrophic organisms co-occurring in oceanic waters. The aim of this project is to study these interactions using *Synechococcus* sp. as the model phototroph (a cosmopolitan and highly abundant member of the picophytoplankton) and *Roseobacter* as the model heterotroph (a dominant and widespread member of the bacterioplankton). Specific metabolites absent from the milieu of *Synechococcus-Roseobacter* co-cultures compared to axenic cultures were identified by LC-MS. One of the compounds (M3) was purified and a proposed chemical formula and substructure were obtained by high resolution MS and NMR. A *Synechococcus* sp. WH7803 null mutant in the gene encoding a type III PKS was constructed. A targeted exometabolomic analysis showed no production of the metabolite in the mutant strain compared to the wild type. Natural product database searches based on MS and preliminary NMR data obtained for the metabolite M3 suggest that it is a novel compound. Further research is required to establish the biological function of the molecule and its biosynthetic pathways. Given the stationary phase peak in production of the compound, we hypothesize that the metabolite is an inter-communication molecule used generally by *Synechococcus* to 'sense' the stationary phase of growth and anticipate senescence.

POSTER PRESENTATIONS - 15

A NOVEL STRATEGY FOR EXPLOITATION OF HOST RNASE E ACTIVITY BY A MARINE CYANOPHAGE

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Previous studies have shown that infection of *Prochlorococcus* MED4 by the cyanophage P-SSP7 leads to increased transcript levels of host endoribonuclease (RNase) E. However, it has remained enigmatic if this is part of a host defence mechanism to degrade phage mRNA or if this single-strand (ss)RNA-specific RNase is utilized by the phage. Here we describe a hitherto unknown means through which this cyanophage increases expression of RNase E during phage infection and concomitantly protects its own RNA from degradation. We identified two functionally different RNase E mRNA variants, one of which is significantly induced during phage infection. This transcript lacks the 5'UTR, is considerably more stable than the other transcript, and is likely responsible for increased RNase E protein levels during infection. Furthermore, selective enrichment and *in vivo* analysis of double-stranded (ds)RNA during infection revealed that phage antisense (as) RNAs sequester complementary mRNAs to form dsRNAs, such that the phage protein-coding transcriptome is nearly completely covered by asRNAs. In contrast, the host protein-coding transcriptome is only partially covered by asRNAs. These data suggest that P-SSP7 orchestrates degradation of host RNA by increasing RNase E expression while masking its own transcriptome from RNase E degradation in dsRNA complexes. We propose that this combination of strategies contributes significantly to phage progeny production.

POSTER PRESENTATIONS - 16

FLAVOBACTERIIA FROM THE NORTH SEA

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Flavobacteriia are abundant in the open ocean and coastal seas. We characterized the culturable diversity of flavobacteria from the North Sea[1,2], an epeiric sea on the continental shelf of Europe, as this large abundance of marine flavobacteria has so far not been reflected by a high number of strains in culture collections. Cultivation with a new medium, incubation at low temperatures and with long incubation times, and colony screening by a *Flavobacteriia-Cytophagia*-specific PCR detecting 16S rRNA gene sequences led to a collection of phylogenetically diverse strains. Within the *Flavobacteriaceae* strains obtained, 20 isolated strains presumably represented seven novel candidate genera and 355 strains affiliated with 26 of 80 validly described marine *Flavobacteriaceae* genera, based on a genus boundary of 95.0% 16S rRNA gene sequence identity. The majority of strains (276) affiliated with 37 known species in 16 genera (based on a boundary of 98.7% 16S rRNA gene sequence identity), whereas 79 strains likely represented 42 novel species in 22 established Flavobacteriaceae genera. Pigmentation, iridescence, gliding motility, agar lysis, and flexirubin as a chemical marker supported the taxonomy at the species level. This study demonstrated for phylogenetically diverse *Flavobacteriia* originating from the North Sea a cultivability on solid medium. Samples were taken from a diatom bloom in spring 2010 and strains were obtained using dilution cultivation in an artificial seawater medium with micromolar substrate and nutrient concentrations. Flow cytometry demonstrated growth from single cells to densities of 10^4–10^6 cells ml^{-1} and a cultivability of 35%. Novel *Formosa* and *Polaribacter* strains were isolated and had 16S rRNA gene sequence identities of > 99.8% with bacterioplankton sequences of spring or summer 2009. These studies revealed that a large diversity of *Flavobacteriia* can be cultivated. Dilution cultivation in combination with taxon-specific PCR screening assays allows the targeted isolation of sequence-defined taxa. Many strains of this study are now genome-sequenced, to understand the succession of bacterioplankton populations following the annual North Sea diatom spring bloom[3-5].

1 R.L. Hahnke and J. Harder (2013) Phylogenetic diversity of Flavobacteria isolated from the North Sea on solid media. Systematic and Applied Microbiology 36, 497-504

2 R.L. Hahnke, C.M. Bennke, B.M. Fuchs, A.J. Mann, E. Rhiel, et al. (2015) Dilution cultivation of marine heterotrophic bacteria abundant after a spring phytoplankton bloom in the North Sea. Environmental Microbiology 17, 3515-3526, doi: 10.1111/1462-2920.12479 (Epub 2014 May 5)

3 H. Teeling COGITO (Coastal Microbe Genomic & Taxonomic Observatory) http://genome.jgi.doe.gov/COGCoabservatory/COGCoabservatory.info.html

4 H. Teeling, B.M. Fuchs, D. Becher, C. Klockow, A. Gardebrecht, C.M. Bennke, et al. (2012) Substratecontrolled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336: 608–611.

5 H. Teeling, B.M. Fuchs, C. Bennke, K. Krüger, M. Chafee, et al. (2016) Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. eLife 2016;5:e11888

POSTER PRESENTATIONS - 17

MIRRI – THE MICROBIAL RESOURCE RESEARCH INFRASTRUCTURE

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Placed on the European Strategy Forum on Infrastructure (ESFRI) roadmap in 2010 and now aiming for the establishment of a legal structure, MIRRI brings together more than 40 European public Culture Collections and microbial domain Biological Resource Centres (mBRCs). Covering the whole spectrum of microorganisms, the consortium holds more than 400,000 microbial resources. The overall objective of MIRRI is to create a quality-driven, decentralised centre for genetic resources and information to facilitate access to microbial material within Europe. This idea of a European One Stop Shop to serve users' needs does not only comprise a significantly broader range of offered raw material with ensured legal compliance (Nagoya Protocol and underlying international and national regulations), but also access to associated data as well as to expert knowledge in fields of microbiology. A special focus lies on improved collaborations with and support of the European bio-industry. It is envisaged that removing existing obstacles such as yet under-utilisation of natural microbial resources and the diminishing expertise in their isolation and characterization, will be of benefit for the development of the European bio-economy. A second major intention of MIRRI is to establish a platform for capacity building. This includes not only transnational access to facilities but also a master curriculum for students in microbial systematics and resource management. By offering tailor-made training in the omics area and in taxonomy MIRRI will fight against the increasing loss of expertise. These well-trained next-generation scientists are necessary to work with bio-industry in Europe to accelerate the discovery processes and innovations. Although not yet fully operable, basic services from MIRRI partners are available. For details please visit www.mirri.org/user-service.

POSTER PRESENTATIONS - 18

THE ECOLOGY AND EVOLUTION OF SECONDARY METABOLISM IN A MODEL GROUP OF MARINE BACTERIA

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Bacterial secondary metabolites are the biosynthetic products of large and complex gene clusters. These clusters are well suited for horizontal gene transfer, with acquisition events providing immediate opportunities to test small and often biologically active molecules for their effects on fitness. Those that provide a selective advantage are maintained while those that do not are lost. These acquisition events are mediated by the availability of gene clusters in the "local gene pool" and can spread through globally distributed populations. The marine actinomycete genus *Salinispora* has become a useful model to study the ecology and evolution of secondary metabolism. Based on 120 genome sequences, we have observed high rates of pathway acquisition and exchange. More than 50% of the gene clusters occur in only one or two strains, suggesting they were recently acquired. These gene clusters largely occur in two of 21 genomic islands, suggesting a yet to be defined mechanism that directs them to specific chromosomal sites. Gene clusters that become fixed within a species are observed to migrate into more conserved regions of the core genome suggesting they are no longer available for exchange. Transcriptomic analyses revealed that more than half of the gene clusters are silent under normal laboratory growth conditions. Comparative analyses indicate subtle regulatory differences among related gene clusters that explain why some clusters will remain permanently silent. These observations suggest that regulatory element deletion represents an alternative or intermediate strategy to gene cluster loss. Comparative "omics" are providing unique opportunities to infer the evolutionary histories of biosynthetic gene clusters independent of the bacteria in which they reside and to understand the processes that generate secondary metabolite structural diversity.

POSTER PRESENTATIONS - 19

THE EUROPEAN MARINE BIOLOGICAL RESOURCE CENTRE: A PAN-EUROPEAN RESEARCH INFRASTRUCTURE CONSORTIUM (EMBRC-ERIC)

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The EMBRC-ERIC is a pan-European distributed research infrastructure that aims to provide a strategic delivery mechanism for excellent and large-scale marine biological and ecological research in Europe. With its services, EMBRC will support both fundamental and applied research based on marine bioresources and marine ecosystems. In particular, EMBRC aims to drive forward the development of blue biotechnologies. Catering for users from academia, industry, policy and the technology sectors, EMBRC provides a unique entry point to access an integrated portfolio of services, bio-technology research platforms, marine organisms, as well as analytical and historical environmental data, to investigate the potential of the marine to deliver for societally relevant research domains such as biomedicine, sustainability of food production, industrial process innovation, and environmental adaptations to climate and pollution.

POSTER PRESENTATIONS - 20

EMBRIC - DISCOVERY AND EXPLOITATION OF NOVEL MARINE PRODUCTS AND BIOMOLECULES: "EXPERTISE ON MICROBIAL RESOURCES"

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Biological Resources, such as microorganisms and their derivatives, are the essential raw material for the advancement of Biotechnology, Human health and Research and Development in the Life Sciences. The microbial domain Biological Resources Centres (mBRCs) aim at facilitating the deposit of important microbial materials and improving access to enhanced quality microbial resources that drive scientific excellence and Research and Innovation. The Biological Resource Centre of Institut Pasteur (CRBIP) maintains a broad diversity of prokaryotic species; strains containing plasmids and genes of interest, strains having particular properties, etc. The CRBIP distributes its biological resources in France and abroad following safety standards for health and the environment in compliance with regulations and laws (Best practices, Nagoya, Protocol, etc.) and ensuring maximum traceability. In addition, it is involved in different research projects focused on bacterial genomics and proteomics, as well as on preservation. The CRBIP is organized to improve the access to microbial material and in particular to bacterial resources, in order to enhance exchange within Europe and non-European countries, to facilitate cross-disciplinary experimental work, to innovate with new better-performing methodologies such as collaborations between private and public laboratories. At the European level, the CRBIP is involved in the MIRRI infrastructure and in the EMBRIC project. Furthermore, the CRBIP offers services such as the identification, characterization and preservation of newly discovered strains, which will, in turn, drive biotechnological research, allowing generating new data to complement the data sets of strains of high relevance for research and development; and the Next Generation Sequencing (NGS), which is used in order to identify genes and regulatory elements involved in different metabolic processes, providing a wealth of knowledge for comparative biology studies through whole genome sequencing of a wide variety of organisms which could serve as a source of novel biomolecules.

POSTER PRESENTATIONS - 21

THE WORKING CLASS HEROES OF MICROBIAL MATS

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Different types of coastal microbial mats that are influenced by both seawater and freshwater develop along the tidal gradient of the North Sea beach of the Dutch barrier island Schiermonnikoog. They form nearly closed micro-scale ecosystem and typically consist of three characteristic layers: a green layer mainly composed of filamentous cyanobacteria, a purple layer composed of purple sulphur bacteria and a black layer indicative for active sulfate reduction. Despite the apparent simple organization, 16S ribosomal RNA gene based amplicon sequencing revealed that these mats harbor a huge microbial diversity and a large metabolic and biotechnological potential. However, DNA based analysis does not necessarily provide information on the active fraction of the microbial community. Hence, we focused on the active microbial community by comparing 16S rRNA gene sequences obtained from the DNA fraction with sequences obtained from the rRNA pool itself. Moreover we sampled at different locations and time points during a 24-hour period to study potential spatial and temporal variations and compared different primer sets for Illumina based 16S rRNA amplicon sequencing. Here I present an optimized workflow and bioinformatics pipeline to infer the resident and active community composition in three different stations during three seasons of the year. Furthermore I will discuss the surprising finding that unicellular rather than the visual dominant filamentous Cyanobacteria form the majority of the active community.

POSTER PRESENTATIONS - 22

NEW ENZYMES AS BIOCATALYSTS FROM MARINE MICROORGANISMS:

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Marine bacteria (from UMIL) and marine yeasts (from UBO) were screened for enzymes with potential as biocatalysts. Cultures were grown in media with increasing NaCl concentrations (0-10%) for simultaneously testing growth and enzymatic profiles. The supernatants were all processed for obtaining crude extracts where polysaccharides can be evaluated. Four enzymatic activities were screened: carboxylesterases (EST), ω-transaminases (ω-TA) ketoreductases (KRED), and fructofuranosidases (FF). A screening among bacterial strains was carried out for the resolution of racemic propyl ester of anti-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid 1, a key intermediate for the synthesis of D-cloprostenol. *Bacillus horneckiae* 15A gave highly stereoselective reduction of (*R,S*)-1, whereas *Halomonas aquamarina* 9B enantioselectively hydrolysed (*R,S*)-1; in both cases, enantiomerically pure unreacted (*R*)-1 could be easily recovered and purified at molar conversion below 57– 58%, showing the potential of marine-derived enzymes in stereoselective biocatalysis. A strain of *Virgibacillus pantotheticus* (21D) showed optimal growth and activity in the presence up to 10% NaCl concentration, showing both enantioselective esterase and ketoreductase activity. This strain showed also high ω-transaminases activity and its genome was sequenced. Three enzymes (KRED1-*Vpan*, EST1-*Vpan* and TAM1-*Vpan*) were produced as recombinant proteins in *E. coli* and are currently used for evaluating their potential as biocatalysts. Marine yeasts from UBO were used for evaluating KRED activities. In this case, we used a seawater-based marine medium for the growth and resting cells for the stereoselective reduction of 6 substrates, two of them are key-intermediates for chemoenzymatic synthesis of pharma ingredients (pramipexole and levogestrel). The best results were achieved with a strain of *Meyerozyma guilliermondii* and one of *Rhodotorula mucilaginosa*, which gave complementary and good enantioselectivity. Interestingly, these strains can be used in seawater also for the bioconversion. Finally, yeasts were used for fructooligosaccharides (FOS) production from sucrose (fructofuranosidases activity). No significant results could be observed, even if different conditions were applied. The screening was extended to other newly isolated halophilic fungi; a strain of *Cladosporium cladosporioides* resulted an efficient producer of fructooligosaccharides (FOS) from sucrose, giving mainly 1-kestose, 1-nystose, 1 F-fructosylnystose, 6-kestose, neokestose, and also blastose, an unconventional disaccharide. Lyophilized mycelium of *C. cladosporioides* grown in a seawater-based medium produced 344 g/L (57% w/w) of total FOS from 600g/L of sucrose in seawater under optimized conditions after 72h. Most of the bioprocesses studied were entirely accomplished in seawater.

POSTER PRESENTATIONS - 23

EXPLORING THE SPONGE HOLOBIONT DYSIDEA AVARA WITH MOLECULAR TOOLS AND BY MICROBIAL CULTIVATION

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Introduction: Marine sponges (*Porifera*) belong to the most ancient metazoan animal phylum, dating back approximately 600 million years into pre-Cambrian times. These active filter feeders are currently known for their ability to produce a high diversity of secondary metabolites as a means of chemical defence. A high number of these compounds are of interest to the pharmaceutical industry due to their anti-viral, anti-cancer or antimicrobial activities. It is now generally accepted that microorganisms can make up to 40% of the sponges biomass and live permanently associated with the sponge. Cultivation of sponges and their associated microbes has been in focus for some time as these organisms have the potential to constitute 'biological fermenters' for the production of interesting compounds. However, an immortal sponge cell line has yet to be established, and the cultivation of the microbial symbionts has, in many cases, not been achieved. In this study we investigate the bacterial community associated to the Mediterranean sponge *Dysidea avara* by culture independent methods and aim at increasing the cultivable fraction of key symbionts.

Methods: Sponge cell suspensions and the corresponding bacterial fraction were sequenced using 16S rRNA gene community profiling on an Illumina MiSeq platform. Sequencing results were analyzed using modified versions of the QIIME pipeline. OTUs were picked based on 100% sequence identity. Sponge cell suspension was plated on undiluted and ten times diluted marine agar and Mueller Hinton agar with and without the addition of 6 antibiotics. Inoculated plates were incubated with and without natural light for three weeks. Colonies were picked from all conditions and identified by 16S rRNA gene sequencing using Sanger. Comparison between cultivation dependent and cultivation independent data was conducted using nBLAST considering only hits with ≥99% sequence identity.

Results: Amplicon sequencing resulted in 277 OTUs and revealed that the D. avara associated bacterial community was dominated by the phyla *Proteobacteria* (63%), *Bacteroidetes* (19%) and *Cyanobacteria* (6%). More than 70% of all sequences were comprised of only 30 highly abundant OTUs. The cultivation experiment yielded 37 isolates and included two novel *Flavobacteria* strains with less than 97% sequence identity to the closest cultured isolate. Furthermore, *Pseudovibrio* and *Ruegeria* species were isolated. Comparison to amplicon data revealed that 5 out of the top 24 OTUs were retrieved in culture which reflects 15% of the *D. avara* associated bacterial community based on relative abundance data.

Conclusion: Although *D. avara* is considered a low microbial abundance sponge, we conclude that this sponge associates to a distinct bacterial community which differs from the surrounding seawater. Furthermore, the overlap between amplicon sequencing data and OTUs retrieved in cultivation suggests that we can now study a significant part of the symbiotic community *in vitro*. This opens new perspectives for co-cultivation approaches in future attempts towards sponge cell line development.

POSTER PRESENTATIONS - 24

DEEP MIXING AND MICROBIAL GENE EXPRESSION IN THE GULF OF AQABA / EILAT

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The water column in the Gulf of Aqaba is characterized by summer thermal stratification and deep winter mixing. During the mixed winter period, temperature, salinity, oxygen, and nutrient profiles are generally uniform throughout the water column, while irradiance is the preeminent environmental factor changing with depth. Vertical mixing currents are expected to homogenize the distribution of planktonic cells. To characterize the microbial community under these unique conditions, we compared gene expression and community composition between the photic and the aphotic zone. Euryarchaeota were the most abundant group with 24 – 35% of all 16S sequences. SAR11 comprised a higher fraction of 16S sequences at the surface, *Flavobacteriia* and *Rhodospirillales* at depth, possibly as a result of higher nutrients load at the deep sample, injected to the mixed layer from the deep layer. Cyanobacteria and phototrophic microalgae presented substantial transcriptional activity in the aphotic sample, with a different gene suite expressed than at the surface. While autotrophic surface transcripts were enriched in photosynthetic and light induced genes, aphotic transcripts were characterized by genes involved in glycogen and amino acid biosynthesis, regulation of gene expression and replication. The most abundant cyanobacterial transcripts, especially at depth, were of the ncRNA family Yfr103. Microbial gene expression implied competition over nutrient resources with high expression of ammonium transporters both in SAR11, *Synechococcus* and Thaumarchaeota. Euryarchaeal transcripts were characterized by transporters of amino acids and other organic compounds, suggesting niche partitioning between *Thaumarchaeota* and *Euryarchaeota* regarding nitrogen sources. Partitioning was also observed for phosphorus acquisition with *Thaumarchaeota* expressing phosphonate ABC transporters and *Euryarchaeota* expressing inorganic phosphate transporters. Our results suggest distinct gene expression in the photic and aphotic zone of the mixed water column. Vertical mixing adds a layer of complexity to gene expression patterns, which in part respond to external cues that are modified by mixing.

POSTER PRESENTATIONS - 25

RECOMBINANT COLLAGENASES AND MARINE ORIGIN PROTEASES IN BIOTECHNOLOGICAL APPLICATIONS

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Recently biomolecules and biomaterial from marine resource has been investigated for biotechnological application, in particular in regenerative medicine. Proteases play an important role in the field of tissue dissociation. Collagenases class I (CI) and class II (CII) currently available for tissue dissociation are produced from *Clostridium histolyticum* (human pathogen) strains. ABIEL, Spin off of University of Palermo and IAMC- CNR, recently produced the collagenase class I and II using the recombinant DNA technologies (PCT WO 2011/073925 A9). Using a synergic approach based on functional biochemical fractionation and computational analysis, we purified and identify enzymes from marine organisms and bacteria in particular marine bacterial belonging to the *Vibrio* family. Their activity was characterized and they were used to accelerate the digestion of extracellular matrix. We compared their activity with Neutral protease from *C. histolyticum* or Thermolisin from *Bacillus thermoproteolyticus* rocco that exert a critical role in the pancreatic tissue dissociation for islets release. Additionally, we used several in vitro procedures together with ex vivo mouse tissue dissociation assessment, to evaluate the dissociation activity[1]. As a result enzyme activity, purity, and formulation strongly influence the outcome of the cells isolation. To date such factors represent the most critical steps on preparation of enzymes.

References

1 Salamone M. et al. *PLoS ONE*. 2015: e0126349. doi:10.1371/journal.pone.0126349

POSTER PRESENTATIONS - 26

THE MARINE BIOTECHNOLOGY ERA-NET

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The Marine Biotechnology ERA-NET (ERA-MBT) is a consortium of 19 national funding agencies seeking complementarities between national activities by pooling resources to undertake joint funding of transnational projects in the area of marine biotechnology. The activities including the development of a Strategic Roadmap for marine biotechnology will support the European bioeconomy. Europe's marine ecosystems and organisms are largely unexplored, understudied and underutilized, in spite of Europe's access to an extensive and diverse set of marine ecosystems which support enormous biodiversity. This resource, through the coordinated application of marine biotechnology, has the potential to provide a major contribution towards addressing the Grand Challenges of our future. The ERA-MBT vision is to support Europe's marine biotechnology community to participate in a lasting enterprise-driven network that adds value to marine biological resources in ways that nurture and sustain the lives of European citizens.

POSTER PRESENTATIONS - 27

DETERMINATION OF THE MARINE MICROBIAL DIVERSITY AND ECOLOGICAL DYNAMICS IN THE EQUATORIAL EASTERN PACIFIC

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The Easter Equatorial Pacific, where the Galapagos Islands are located, is the meeting point of two large marine ecosystems: the Tropical East Pacific Ecosystem in the north, characterized by warm waters, and the Humboldt Current System which consist of cold, nutrient-rich, waters coming from the south. This confluence produces some of the most dynamics marine environments with strong seasonal changes, which are further stretched by regional events as the El Niño and La Niña. This makes this region a very interesting area for the study of the diversity, ecological dynamics and the isolation of microorganisms with potential biotechnological applications. However the neighboring countries lack the infrastructure and human resources for a comprehensive study of this region. Here I propose the development of the marine microbiological research capacities of Ecuador, a country neighboring area and in charge of the Galapagos Islands. By launching the first national research program in the topic, which aims to take advantage of the infrastructure and seafaring capacities already in the country, is expected to conduct identification (genomic), inventory and determination of the ecological dynamics of the microorganisms in the region.

As a first exploratory step the program aims to:

1. Establish an inventory of microbial genetic diversity (algae, bacteria, fungi and viruses) in the Easter Equatorial Pacific.
2. Identify the biotechnological potential of the microorganisms in the area through isolation and culture.
3. Determine the spatial dynamics, ecological relationships and seasonal variability of microbial communities of the Easter Equatorial Pacific.
4. Strengthen high-level academic training in genetics and marine biotechnology in Ecuador.
5. Strengthen national research capabilities in the field of genetics and marine biotechnology through technology transfer mechanisms.

I think that, although ambitious, these objectives can be achieve by the formation if a core research group with the collaboration of international partners and a not so elevated initial investment in research equipment. My current institution has already compromised resources to the present proposal and I expect to reach to a wider international audience in order to form strategic alliances for the undertaking of this project.

POSTER PRESENTATIONS - 28

MARINE MICROORGANISMS FOR INNOVATIVE BIOPOLYMERS: EPS AND PHAS

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The ocean harbors a vast variety of marine organisms that are diverse in their physiology and adaptations. The marine environment represents a largely unexplored source for isolation of new microorganisms (bacteria, fungi, microalgae-cyanobacteria and diatoms) that could be the main producers of innovative biopolymers.

Polymaris developed a strong and unique expertise in research, characterisation, scale up processes development and commercialization of innovative marine biomolecules such as the bacterial exopolysaccharides (EPS) and polyhydroxyalkanoates (PHAs).

Some bacteria can synthesize diverse structural, functional and valuable polysaccharides and PHA.

Bacterial exopolysaccharides (EPS) are synthesized and secreted into the external environment. These biopolymers are not carbon source related. Each microorganism will synthesize a unique molecule which cannot be chemically synthesized.

PHAs are polyesters consisting of hydroxyalkanoic acids that are linked through ester bonds between the hydroxyl group and the carboxylic group of the next monomer. These natural biopolyesters are accumulated by some bacteria in stress conditions when an excess of carbon is available in the environment and when at least one other nutrient is stoichiometrically limiting growth. The main physiological role of PHAs is as a storage of carbon and energy in the form of intracellular granules.

These innovative natural and biodegradable biomolecules are produced using private and original microorganism collection strains isolating from the huge marine biodiversity collected during oceanographic expeditions.

Polymaris established controlled production routinely but also develops constantly news processes thanks to pilot-scale bioreactors. An innovative technical platform and state-of-the-art equipment allow the production of a few hundred milligrams to several kilograms of pure molecules.

Polymaris is an interdisciplinarity company which can complete the research and development of specialised needs and their commercialization in biopolymer field.

During the project MaCuMBA, Polymaris brought its expertise in the screening, the production and the characterisation of new Biopolymers. This Biopolymers were produced from marine bacteria discovered by the other partners.

POSTER PRESENTATIONS - 29

EMBRIC, A DOOR TOWARDS MARINE BIOTECHNOLOGY INNOVATION

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The EMBRIC (European Marine Biological Resource Infrastructure Cluster) project is a cluster of six research infrastructures (RI) joint with the objective of fostering innovation in marine biotechnology. The cluster is designed to propose integrated multidisciplinary value chains of services for the exploration of marine bioresources and their sustainable exploitation as sources of biomolecules and/or as whole organisms for food. The cluster unites RIs that provide access to the full spectrum of diversity of marine organisms (EMBRC) or are specialized in the provision of specific groups of organisms (MIRRI: prokaryotes and fungi; AQUAEXCEL: finfish). Using these biological resources as raw materials, the cluster is developing service-oriented workflows for natural product discovery and for genetic selection in aquaculture. EU-OPENSCREEN contributes its services and expertise in the area of natural product discovery, while AQUAEXCEL does likewise in the aquaculture domain. ELIXIR provides cross-cutting expertise on data services and management. Finally, to establish the technology transfer identity of EMBRIC, RISIS provides its expertise in the analysis of innovation ecosystems.

The EMBRIC cluster mission is to build interconnectivity along three dimensions: science, industry, and regional RDI policies. This mission will be accomplished by developing integrated workshops of high quality services for access to biological, analytical and data resources, and deploy common underpinning technologies and practices; strengthening the connection of science with industry by engaging companies and by federating technology transfer services; and engaging RDI policy-makers with the aim of consolidating a perennial pan-European virtual infrastructure cluster rooted in the maritime regions of Europe.

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