ABSTRACTS:
Chemical Ecology of Marine Holobionts:
Oral Presentations

In programme order
Session 2

Infection and resistance in complex microbial communities of the plankton - small scale processes with global impact

Georg Pohnert, **Marine Vallet, Tim U.H. Baumeister, Kathleen Thume, Emilio Cirri, Nils Meyer, Arite Bigalke**

Friedrich Schiller University Jena, Germany; Institute for Inorganic and Analytical Chemistry; Department for Bioorganic Analytics; email: Georg.Pohnert@uni-jena.de

Planktonic communities are complex microbial assemblages with continuous changes in species composition. Seasonal species succession is highly predicable but the underlying regulative principles are only poorly understood. We hypothesize that chemical mediators released by the interacting partners explain a substantial part of the community dynamics. Here we introduce how chemical signals and defensive metabolites shape the interaction of unicellular algae and pathogens in the plankton. Flagellated oomycetes frequently infect unicellular algae, thus limiting their proliferation. Using single cell mass spectrometry, bioassays and infection models we show how the marine oomycete *Lagenisma coscinodisci* rewires the metabolome of the bloom-forming diatom *Coscinodiscus granii*. The algal alkaloids β-carboline and 2,3,4,9-tetrahydro-1H-beta-carboline-3-carboxylic acid are induced during infection and support the success of the pathogen. Single-cell profiling with AP-MALDI-MS and confocal laser scanning microscopy reveals that algal carbolines accumulate in the reproductive stages of the parasite. The compounds arrest the algal cell division, increase the infection rate and induce plasmolysis in the host. In another example we show how infection of algae with lytic bacteria is dependent on dynamic induction of resistance factors using approaches from single cell resolution up to ecosystem wide manipulations in mesocosms.

Keywords: Single Cell Mass Spectrometry, Metabolomics, Oomycete Infection, Diatoms, Bioassays

Anthraquinones produced by crinoids allow host selection for the symbiotic snapping shrimp *Synalpheus stimpsonii*

A Lourtie[1,2], G Caulier[1], L Brasseur[1], J Delroisse[1], L Terrana[1], P Gerbaux[3], P Flammang[1], J Mallefet[2], I Eeckhaut[1]

[1] University of Mons - UMONS, Research Institute for Biosciences, Biology of Marine Organisms and Biomimetics Unit. 23 Place du Parc, B-7000 Mons, Belgium.
[2] University Catholic of Louvain – UCLouvain, Earth and Life Institute, Marine Biology Laboratory. 3 Place Croix du Sud, bt 4, B- 1348 Louvain-la-Neuve, Belgium
[3] University of Mons - UMONS, Research Institute for Biosciences, Organic Synthesis and Mass Spectrometry Laboratory. 23 Place du Parc, B-7000 Mons, Belgium.

Quinones are molecules produced by various organisms, including feather stars (Echinodermata, Crinoidea). Besides being one of the major pigment groups that provide their bright colors, they can also act as defensive molecules that make crinoids unpalatable and even repellent to most organisms. Conversely, these organisms are also known to shelter an important associated fauna, such as the ectocommensal snapping shrimp *Synalpheus stimpsonii*. Around the Great Reef of Toliara (Madagascar), *S. stimpsonii* can be found in association with two different species of crinoids: *Comanthus wahlbergii* and *Phanogenia distincta*. Our study investigated the chemical cues that allow host selection for this symbiont. The chemical attractiveness of the two crinoid hosts and a non-host species, *Cenometra bella*, was tested in an olfactometer. The three crinoids released cues in the ecosystem that were attractive for *S. stimpsonii*. Analyses of purified *P. distincta* extracts by mass spectrometry highlighted three kinds of anthraquinones (*i.e.* rhodoptilometrin, 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone and a new crinoid anthraquinone). This mixture of anthraquinones seems specific to this species, as suggested by the current literature. Moreover, when tested in the olfactometer, these extracts induced a similar attractive behavior on the shrimp. Same results were also observed with an addition of pure commercial anthraquinones. Therefore, we suggest that crinoid anthraquinones are not confined to their traditional defensive function, but also act as kairomones involved into the host selection in the symbiotic association between *S. stimpsonii* and *C. wahlbergii* or *P. distincta*.

Keywords: Marine chemical ecology; Kairomones; Feather stars; Olfactometry

A multi-omics approach deciphers how temperature and copper stress shape seaweed-microbiota interactions at the surface of *Taonia atomaria*

Benoit Paix[1], Nicolas Layglon[2], Christophe Le Poupon[2], Sébastien D'Onofrio[2], Benjamin Misson[2], Cédric Garnier[2], Gérald Culioli[3] and Jean-François Briand[1]

[1]Université de Toulon, Laboratoire MAPIEM, EA 4323, Toulon, France
[2]Université de Toulon, Aix Marseille Université, CNRS, IRD, Mediterranean Institute of Oceanography (MIO), France
[3]Université d'Avignon, CNRS, IRD, Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), France

Although considered as holobionts, macroalgae and their surface microbiota share intimate interactions that are still poorly understood. In this context, the epibacterial communities of the Ochrophyta *Taonia atomaria* exhibited a high specificity whatever the five contrasted sites investigated on the North West Mediterranean coast. Using a multi-omics approach combining metabarcoding and metabolomics, the holobiont dynamics was found to vary as a whole in agreement with previous studies. During the occurrence period of *T. atomaria*, epibacterial densities and α-diversity increased while the relative proportion of core and specific communities decreased. Pioneer bacterial colonizers (e.g. *Litorimonas* and *Granulosicoccus* genera) constituted a large part of the specific and core taxa and displayed potential functional features involved in adhesion, biofilm formation and adaptation to the epiphytic lifestyle. Then, the concomitant increase of temperature and several algal compounds, especially dimethylsulfoniopropionate could explain the bacterial diversification, especially with *Roseobacter* taxa specialized in the catabolism of this metabolite. Copper concentration constituted a second factor shaping the holobiont system. The resulting oxidative stress caused an adaptation of the algal surface metabolome with a higher expression of photo-pigment including chlorophyll derivatives and carotenoids (β-carotene and fucoxanthin), which could result in the selection of particular epibacterial taxa. In a context of global change, this study brought new insights on the dynamics of a Mediterranean holobiont submitted to heavy anthropic pressures.

Keywords: Holobiont; macroalgae, metabolomics; metabarcoding; environment

Exploring the microbiota of *Saccharina latissima*

Burgunter-Delamare Bertille [1], Rousvoal Sylvie [1], Tanguy Gwenn [2], Legeay Erwan [2], Fredriksen Stein [3], Diehl Nora [4], Bischof Kai [4], Boyen Catherine [1] and Dittami Simon [1]

[1] Sorbonne Université, CNRS, UMR 8227 – Laboratory of Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, 29688 Roscoff, France
[2] Sorbonne Université, CNRS, FR2424, Station Biologique de Roscoff, CS 90074, 29688 Roscoff, France
[3] University of Oslo, Department of Biosciences, PO Box 1066, Blindern, N-0316 Oslo, Norway
[4] Marine Botany- BreMarE, FB 02, University of Bremen, Bremen, Germany

Saccharina latissima is an abundant kelp-forming species of brown macroalgae in Europe. Its current distribution ranges from Spain to Spitzbergen, although several populations are in decline. Despite its economic and ecological importance, little is known about the composition of its microbiome and the interactions of the latter with the host. To explore the microbiome of *S. latissima* and to determine if specific patterns in microbial communities are correlated with algal fitness, we sampled both healthy and diseased (signs of degradation, twisted stipe, etc.) individuals from different geographic locations (Brittany, Helgoland, Southern Norway, and Svalbard) and in different seasons. For each sampled individual, we assessed the microbiome composition by 16S metabarcoding and isolated live microbial cultures for future co-culture experiments. Amplicon Sequence Variant (ASV) analyses highlighted the *Proteobacteria* (*Alpha*- and *Gammaproteobacteria*) as dominant phylum and showed a separation between the apex and meristem bacterial communities for all samples. Furthermore, several ASVs were specific to either healthy or diseased individuals. Bacterial cultures (381 isolates in total, belonging to four phyla, 16 orders, 45 genera) were dominated by *Firmicutes* and *Gammaproteobacteria*. Among these isolates, 48 were found exclusively on diseased individuals and 37 exclusively on healthy ones. These data contribute to our understanding of the variability of the *S. latissima* microbiome, establish a basis for ongoing experimental evaluations of algal-bacterial interactions, and serve for comparisons with other model systems such as *Ectocarpus*.

Keywords: Holobiont; brown macroalgae; kelp; metabarcoding

Deciphering the marine algal holobiont interactions for the discovery of new eco-compatible antifoulings

Emilie ADOUANE[1,2], Anne TOURNEROCHE[1], Raphaël LAMI[2], Soizic PRADO*[1]

[1] Muséum National d'Histoire Naturelle, Unité Molécules de Communication et Adaptation des Micro-Organismes (UMR 7245), CNRS, Sorbonne Université, Paris, France
[2] CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, Sorbonne Université, Banyuls-sur-Mer, France

Biofilms are complex communities of microorganisms in contact with a surface and included in a matrix that they secrete. These microbial biofilms provoke many problems for military, industrial, and recreational activities. Most of the used antifouling compounds are particularly toxic biocides that have a significant impact on the natural environment. However, no sustainable, environmentally-friendly alternative has been developed. However, such chemical compounds exist in Nature, and many marine species have developed strategies to protect themselves from biofouling. This is particularly the case of macroalgae that secrete molecules targeting quorum sensing, a mode of intercellular communication involved in the formation of biofilms. The search for “quorum quenching” compounds, mimicking this key mechanism, is thus an innovative strategy to identify new antifouling substances.

The knowledge of the algal holobiont has revealed the existence of microbiota associated with macroalgae capable of effecting the fitness of the host alga. Indeed, the algal epimicrobiota is the place of intense chemical communications, which finely regulates the organization of algal surface biofilms but whose mechanisms have been little studied. However, we have demonstrated that the epimicrobiota of the alga *Saccharina latissima*, a key specie of the northern European coasts, is the seat of the production of chemical mediators capable of inhibiting bacterial QS and by extension inhibiting the formation of biofilms. The molecular dialogue which occurs within the algal holobiont appears thus as an efficient bioinspired target to better decipher chemical interactions within the holobiont and to find new sustainable natural compounds.

Keyword : algal microbiota ; antifouling, chemical communication ; holobiont ; metabolites

Coral larval settlement preferences linked to crustose coralline algae with distinct chemical and microbial signatures

HLJ Jorissen[1], P. Galand[2], I Bonnard[1,3], S Meiling[1,4], D Raviglione[1], AL Meistertzheim[2,5], L Hédouin[1,3], B Banaigs[1,3], CE Payri[6], Maggy M. Nugues[1,3]

1 EPHE, PSL Research University, UPVD-CNRS, USR3278 CRIOBE, 66860 Perpignan, France
2 Sorbonne Université, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques, LECOB, 66500 Banyuls-sur-Mer, France
3 Laboratoire d'Excellence « CORAIL», 98729 Papetoai, Moorea, French Polynesia
4 University of the Virgin Islands, St Thomas, 00802-6004, Virgin Islands
5 Plastic@Sea, Observatoire Océanologique de Banyuls, 66650 Banyuls-sur-Mer, France
6 IRD Nouméa, UMR Entropie, 98848 Nouméa, New Caledonia

The resilience of coral reefs is dependent on the ability of corals to settle after disturbances. While crustose coralline algae (CCA) are considered important substrates for coral settlement, it remains unclear whether coral larvae respond to CCA metabolites and microbial cues when selecting sites for attachment and metamorphosis. This study tested the settlement preferences of an abundant coral species (*Acropora cytherea*) against six different CCA species from three habitats (exposed, subcryptic and cryptic), and compared these preferences with the metabolome and microbiome characterizing the CCA. While all CCA species induced settlement, only one species (*Titanoderma prototypum*) significantly promoted settlement on the CCA surface, rather than on nearby dead coral or plastic surfaces. This species had a very distinct bacterial community and metabolomic fingerprint. Furthermore, coral settlement rates and the CCA microbiome and metabolome were specific to the CCA preferred habitat, suggesting that microbes and/or chemicals serve as environmental indicators for coral larvae. Several amplicon sequence variants and two lipid classes - glycoglycerolipids and betaine lipids - present in *T. prototypum* were identified as potential omic cues influencing coral settlement. These results support that the distinct microbiome and metabolome of *T. prototypum* may promote the settlement and attachment of coral larvae.

Keywords: *Acropora cytherea*; coral-algal interactions; coral recruitment; metabolome; microbiome

Carbon and nitrogen uptake and translocation between the single cell marine protist Acantharia and their symbionts

JS Mansour[1], J Decelle[2], C LeKieffre[2], B Gallet[3], S Le Panse[1], F Not[1]

[1] CNRS and Sorbonne University, UMR7144, Adaptation and Diversity in Marine Environment (AD2M) Laboratory, Ecology of Marine Plankton Team, Station Biologique de Roscoff, Place Georges Teissier, Roscoff, France
[2] Univ. Grenoble Alpes, CNRS, CEA, INRAe, IRIG-LPCV, Grenoble, France.
[3] Institut de Biologie Structurale (IBS), University Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France

Acantharia are ubiquitous, heterotrophic single celled plankton in oceanic waters. Their contribution and roles in ecosystems have previously been underestimated, being elusive due to their broad size range and fragility. Yet, recent studies show that they are major components of the planktic community contributing greatly to, among others, the carbon flux. The difficulties inherent to the study of live Radiolaria make it that very little is known about their physiology. Acantharia are known to form a photosymbiosis with the microalgae *Phaeocystis* sp.. The photosymbiont is heavily modified in the acantharian host. These modifications seem to aim to exploit photosynthetic capabilities. Yet, how much the Acantharia relies on photosynthesis or feeding is not known, nor the metabolic interactions of host and symbiont. Here we aimed to elucidate the metabolic dialogue between these (endo)symbiotic partners. Therefore, we used single cell isolations of Acantharia incubated with stable isotopes of carbon and nitrogen. We then used chemical imaging to visualize carbon (nitrogen) uptake, incorporation, and photosynthate translocation between symbionts and host over time, and the effect of nitrogen (NO_3^- or NH_4^+) thereon. With this, we aim to visualize how carbon is translocated in the holobiont and through which partitions (i.e. host or symbiont). Furthermore, we expect to resolve the capabilities of the host to itself directly take up NO_3^- or NH_4^+, as well as how increased nitrogen affects the carbon assimilation.

Keywords: carbon; plankton; *Phaeocystis*; photosynthates; photosymbiosis

Studies of the oxylipin pathway regulation in brown algal kelps

XING Qikun [1], MARKOV Gabriel [1], ROUSVOAL Sylvie [1], LEJEUNE Emeline [2], LEROUX Cédric [2], CORRE Erwan [2], POTIN Philippe [1], LEBLANC Catherine [1]

[1] Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), Station Biologique de Roscoff, France
[2] Sorbonne Université, CNRS, FR2424, Station Biologique de Roscoff, France

In intertidal zones, brown algal kelps are key components of marine forests, and potential sources of ecologically important chemical compounds. Indeed, similarly to land plants and animals, kelps have been shown to feature inducible metabolic responses upon both abiotic and biotic stress, such as the release of free polyunsaturated fatty acids (PUFAs), oxidized derivatives from both C18- and C20- PUFAs, and aldehydes. However, in the brown algal lineage, their biosynthesis pathway has been hardly explored neither their biological and ecological roles in defense or distance signaling. Preliminary results obtained in our laboratory have suggested that some products of PUFA oxidative pathway such as oxylipins and aldehydes might induce defense reactions against grazers in some kelps. To explore their signaling roles in kelps, different chemicals such as aldehydes (4-Hydroxy hexenal, 4-HHE) and oligoguluronate (GG) elicitors were applied on young kelp plantlets of *Saccharina latissima* and untargeted metabolomic analysis revealed the regulation of several oxylipin-derived compounds. In parallel, large-scale transcriptomic analyses were conducted to identify both molecular short- and longer-term responses in *S. latissima*. RNA-seq data were acquired at four time points (0.5h, 1h, 4.5h and 12h) after application of GG. Preliminary result showed significant gene regulation compared to control, including 7 LOX genes mostly up-regulated after 4.5 hours. This indicated an early activation of oxylipin pathway. Altogether, the integration of metabolic and transcriptomic data will help us to decipher the molecular bases of oxylipin pathway regulation in brown algae.

Keywords: Oxylipins; aldehydes; defense signaling; gene expression; transcriptomics; metabolomics

Exploring chemical diversity in the deep sea: linking bacterial symbionts to changes in mussel metabolome

D Michellod, B Geier, D Jakob, N Dubilier, M Liebeke

Max Planck Institute for Marine Microbiology, Bremen, Germany

Deep-sea symbioses represent an untapped world of chemical diversity. We investigated the metabolome of a variety of deep-sea mussel species to unravel chemical cues mediating the interaction between the partners. All 12 mussel species analyzed rely on intracellular chemosynthetic bacteria for their nutrition. They form single or dual symbioses with sulfide-oxidizing bacteria and methanotrophic bacteria. The mussels were collected at hydrothermal vents and cold seeps in two different oceans. Covering this broad sample diversity enabled us to determine the influence of the symbionts on the mussel metabolome and identify symbiont-induced changes. The LC-MS/MS data was analyzed using multivariate statistical analysis, clustering and classification. The data was further mined using GNPS tools to gain a more comprehensive chemical overview and visualize structural diversity through molecular networking. We followed the detected chemical cues with spatial metabolomics. Our mass spectrometry imaging approach helped to localize partner specific metabolites at a micrometer scale. The combination of bulk and spatial analyses revealed a new group of metabolites specific to mussels harboring a particular symbiont species. The elucidation of the symbionts-induced changes may help us to gain a better understanding of the establishment and maintenance of this beneficial symbiotic association in the deep-sea

Keywords: GNPS; HPLC-MS; Interaction; MALDI-MSI; Symbiosis