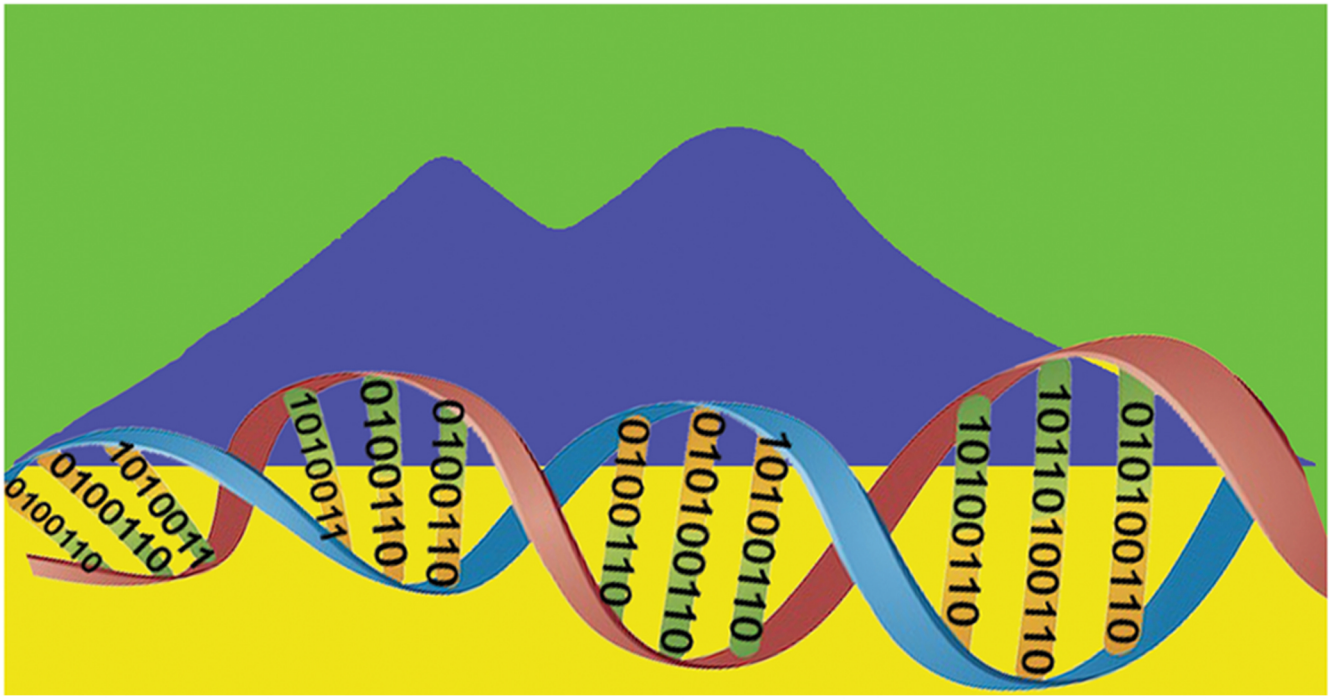
GENOMICS FOR A BLUE ECONOMY

Cutting-Edge Technologies in marine
Natural Products Discovery

BOOK OF ABSTRACTS



11-12 DECEMBER 2019

STAZIONE ZOOLOGICA 'ANTON DOHRN'
NAPOLI, ITALY

Dear colleagues,

I am delighted to welcome you to the workshop "**Genomics for a blue economy"** in Napoli, Italy. The huge biodiversity hosted in the oceans stimulate the research for bioactive molecules from marine bioresources for applications in the field of biotechnology. To exploit these promising biological resources and avoiding impoverishing marine resources, new strategies in the pipeline and a new cohort of cross disciplinary trained scientists are needed. Systems biology approaches, such as genomics, transcriptomics, proteomics and metabolomics, are now being adapted to update natural product discovery platforms. This innovation is in rapid development in terms of sequencing technology, synthetic biology and bioinformatics, with increasing numbers of whole-genome sequences becoming available. Still, a great disparity remains between the genetic potential for natural product production and the actual expression of biosynthetic gene clusters under laboratory culture conditions to produce a biomedically important metabolite. Many genomes appear indeed to possess "silent" or cryptic biosynthetic gene clusters, the products of which appear to be regulated by a variety of environmental factors, and therefore remain largely undetected even by the most sophisticated metabolomics techniques.
The workshop aims at identifying the state-of-the-art of methods, potential challenges, and future directions in the characterization of marine natural compounds and their applications as biotechnological and pharmaceutical products.

Table of Contents

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Workshop Programme

11 December 2019	
8:45 - 9:00	Registration
9:00 - 9:15	**Opening Ceremony**
9:15	**Session 1 - Omics technologies for drug discovery**
9:15 - 10:00	*Keynote* *Computational -omics approaches to natural product discovery* **Prof. Marnix Medema**, Wageningen University
10:00 - 10:30	*Invited* *The biodiscovery effort at IMBM* **Dr. Leonard van Zyl**, The University of the Western Cape
10:30 - 11:00	*Invited* *Use of ion mobility in mass spectrometry-based metabolomics of marine natural products* **Prof. Espen Hansen**, The Arctic University of Norway (UiT)
11:00 - 11:20	**Coffee break**
11:20 - 11:50	*Invited* *Genome editing in diatoms* **Dr. Maria Immacolata Ferrante**, Stazione Zoologica Anton Dohrn
11:50 - 12:10	*Mining metagenomes of Geodia barretti from different depths for biosynthetic gene clusters* **Ms. Asimenia Gavriilidou**, Wageningen University & Reasearch
12:10 - 12:30	*Mining for bioactive products in diatom transcriptomes* **Dr. Valeria Di Dato**, Stazione Zoologica Anton Dohrn
12:40 – 14:00	**Lunch**
14:00 - 14:30	*Invited* *New developments in natural products from marine benthic invertebrates* **Prof. Conxita Avila**, University of Barcelona
14:30 - 14:50	*Bioprospecting Marine Actinomycetes for Novel Anti-Tubercular Drugs* **Dr. Daniela Tizabi**, University of Maryland Center for Environmental Science
14:50	**Session 2 - Improving marine secondary metabolite production**

14:50- 15:35	*Keynote* *Waking-up silent genes for natural product discovery* **Dr. Christophe Corre**, University of Warwick
15:35 - 15:55	*Nutrient starvation to enhance bioactivity in microalgae* **Dr. Chiara Lauritano**, Stazione Zoologica Anton Dohrn
15:55 - 16:10	**Coffee break**
16:10 - 16:30	*Fungal cultivability and activation of silent gene clusters* **Dr. Teppo Rama,** UiT-The Arctic University of Norway
16:30 – 17:15	*Keynote* *Exploring the Microbial Enzymatic Toolbox for Natural Product (Bio-)Synthesis* **Prof. Tobias A. M. Gulder,** Technische Universität München
17:15 - 18:45	**Poster Session – Neapolitan-style Aperitif**

20:00 Social Dinner

Pizzeria Salvo – Via Riviera di Chiaia, 271

12 December 2019

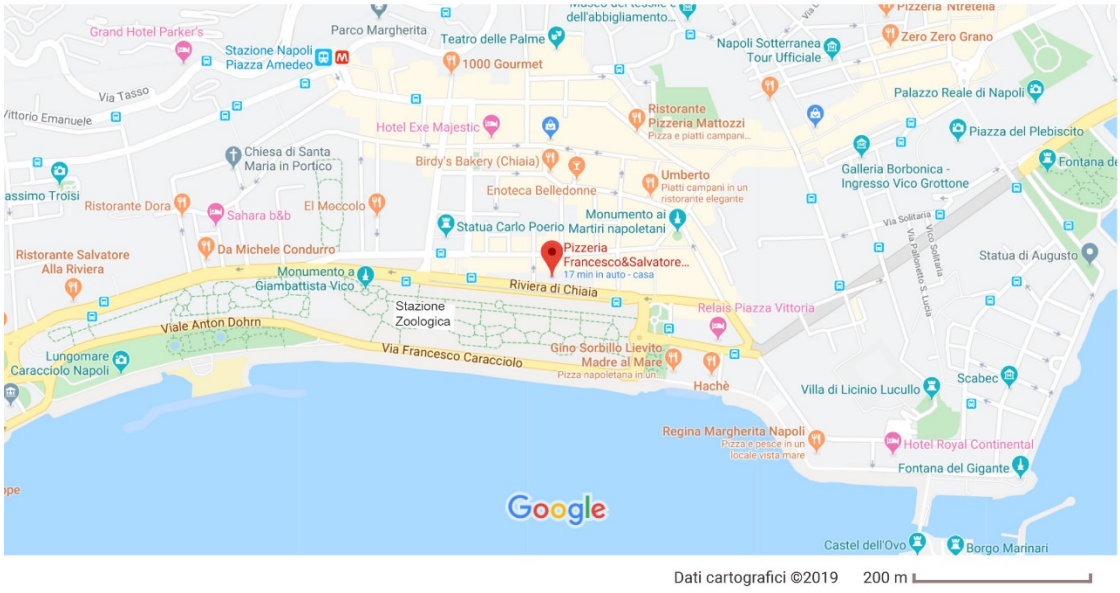
9:15	**Session 3 - An Ocean of big data: which future?**
9:15 - 10:00	*Keynote* *The evolution of genome mining for secondary/specialized metabolite biosynthetic gene clusters* **Prof. Tilmann Weber,** DTU - The Novo Nordisk Foundation Center for Biosustainability
10:00 - 10:20	**Dr. Marco Borra,** Stazione Zoologica Anton Dohrn - The contribute of EMBRC to the Blue Growth
10:20 – 12:45	**Prof. Tilmann Weber,** DTU - Advantages of - omic approaches for natural products discovery **Prof. Marnix Medema** - How to take advantage of the "BIG Data" generated by the –omics technologies.

	Dr. Maria Immacolata Ferrante, Stazione Zoologica Anton Dohrn - The contribute of genome editing techniques **Prof. Ferdinando Boero,** University of Naples Federico II - Sustainability and –omics approaches **Dr. Leonard van Zyl***,* The University of the Western Cape. - The Blue growth in South Africa **Dr. Adrianna Ianora,** Stazione Zoologica Anton Dohrn - New opportunities for sustainable blue growth
12:45 - 13:00	**Concluding Remarks**

Social dinner

The social dinner and party will take place on the evening of the 11th of December at the Pizzeria Salvo

When: Wednesday 11th of December, 8 PM
Where: Pizzeria Salvo, Via Riviera di Chiaia, 271
How to get there: From the Stazione Zoologica, exit from the "Villa Comunale", cross the street and follow Via Riviera di Chiaia to the right, until Via Riviera di Chiaia 271



KEYNOTE AND INVITED LECTURES

Computational -omics approaches to natural product discovery

Marnix H. Medema

Bioinformatics Group, Wageningen University, Wageningen, The Netherlands

Microorganisms produce a wealth of specialized metabolites, which are of great importance from both ecological and clinical perspectives. Due to the accelerated accumulation of omics data, computational methods have become more and more important to identify these molecules and to assess their biological activities. Here, I will highlight the work performed in my research group on using these approaches to accelerate natural product discovery, as well as to study microbe-microbe and host-microbe interactions in human, plant and animal microbiomes. Specifically, I will discuss the use of sequence similarity networking approaches to investigate biosynthetic diversity across large numbers of genomes, and integrative genome/metabolome mining to link gene clusters to molecules

The biodiscovery effort at IMBM

[1]Leonardo Joaquim van Zyl, [1]Dug Rowland, [1]Fazlin Pheiffer, [1]Shanice Adams, [1]Michell Williams, [1]Ross Vermeulen, [2]Antigoni-Angeliki Kyritsi, [1]Marla Trindade

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There is an urgent need for the discovery of new antimicrobial and other bioactive compounds. Marine bacteria, especially those that live in symbiosis with marine invertebrates, have displayed a remarkable ability to produce chemically diverse bioactive compounds compared with terrestrial species. Through IMBM's involvement in 2 EU projects (FP7 PharmaSea and H2020 RISE Ocean Medicines) we have established a collection of highly bioactive marine invertebrate symbionts. Here we present progress that has been made towards uncovering novel multirole compounds from marine invertebrate symbionts using a variety of approaches. These include a bacillomycin D-like compound potentially encoded by a novel pathway, novel NRPS-based siderophore, a new surfactin-like lipopeptide and the potential first report of scalarane-like compounds produced by a bacterium, *Thalassomonas actiniarum*. Following re-classification, the genus *Thalassomonas* now consists of only three species. Our investigation of two of these demonstrated the presence of a high molecular weight molecules with activity against *Echerichia coli* 1699 and *Pseudomonas putida* and low molecular weight compounds active against a melanoma cell line as well as possessing anti-Gram-positive activity. The genomic analysis and activity assays of these strains present a case to support the view that they could rival actinobacteria as source of novel secondary metabolites and could be a rich resource of antimicrobial and other bioactive compounds.

Use of ion mobility in mass spectrometry-based metabolomics of marine natural products

Espen Hansen

Marbio, UiT The Arctic University of Norway, Breivika, N-9037 Tromsø, Norway

Untargeted metabolomics based on mass spectrometry (MS) data is potentially a powerful technique for identifying novel marine secondary metabolites. Modern mass spectrometers have implemented different technical solutions in order to provide MS data with high resolution and high mass accuracy with instruments having sensitivity and large dynamic range. Ion mobility spectrometry (IMS) can be applied in combination with MS in order to improve the quality of fragment data, which again can facilitate the identification of compounds in untargeted metabolomics. A workflow using UHPLC-IMS-MS and untargeted metabolomics for the identification of novel marine secondary metabolites will be illustrated.

Genome editing in diatoms

Maria Immacolata Ferrante

Stazione Zoologica Anton Dohrn, Napoli, Italy

Diatoms are major components of phytoplankton and play a key role in the ecology of aquatic ecosystems. These unicellular algae are of great scientific importance for a wide variety of research areas, including marine ecology and biotechnology. During the last 20 years, genomic information became available first for two model species, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum,* and gradually for additional species each chosen as a model to study a specific process (adaptation to cold environments, tolerance to low iron conditions, increased ability to accumulate lipids, life cycle control and more). Substantial progresses have been made over the years also for genetic manipulation strategies: genetic transformation in diatoms has been available since 1995, allowing to overexpress genes or to silence them using RNA interference, while more advanced methods to obtain gene knockouts, such as tailored TALEN (Transcription Activator-Like Effector Nucleases) and the CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats)/Cas9 system, became available in the last decade. Both systems are based on endonucleases that can induce double strand breaks in the DNA, which subsequently trigger an error-prone DNA repair system that may introduce insertions or deletions, eventually resulting in the inactivation of the targeted gene. A few *P. tricornutum* knockout mutants have been produced to study genes involved in chloroplast functioning and in the nutrient transport process, and our current work involves genetic manipulation of enzymes active in the lipid metabolism.
The availability of these methods in diatoms finally allows to perform genetic manipulations for strain improvement similarly to other traditional and more established systems, thus contributing to establish these algae as a significant, renewable and sustainable resource of biomass for feed, food, energy, and other value-added products.

New developments in natural products from marine benthic invertebrates

Conxita Avila

University of Barcelona, Barcelona (UB)

Recent research in our lab is focusing at new approaches to study the natural products from marine benthic invertebrates. A part of the classical description of new compounds and their bioactivities, we are looking at the production of these metabolites under situations of stress, trying to see changes in gene expression related to natural compounds synthesis. For this, we are now studying a Mediterranean mollusk. On the other hand, we are using CADD (Computer-Aided Drug Design) techniques to help us in the search for bioactivities for our compounds, and experimentally validating these results. For these, we are studying selected tunicate compounds, among others.
In this talk, a summary of these recent developments will be provided.

Waking-up silent genes for natural product discovery

Christophe Corre

University of Warwick, Coventry, UK

Mining bacterial genomes has revealed a vast number of gene clusters proposed to direct the biosynthesis of novel specialized natural products. However, many of these gene clusters remain silent, or are poorly expressed, in laboratory growth conditions.
Our research group is particularly interested in investigating the molecular mechanisms by which pathway-specific transcriptional regulators control the expression of silent and cryptic biosynthetic gene clusters.
By exploiting our structural and functional understanding of specific classes of transcriptional regulators, we have successfully uncovered novel families of natural products and, subsequently, novel biocatalysts.
This presentation will first introduce some of the fundamental work we have carried out before discussing the specific strategies we have used to access the metabolic products of silent biosynthetic gene clusters.

Exploring the Microbial Enzymatic Toolbox for Natural Product (Bio-)Synthesis

Tobias Alexander Marius Gulder

Technical University of Munich, Germany

Microbial natural products serve as one of the main sources of novel chemical scaffolds for biomedical applications. Many of these compounds have highly complex molecular architectures that are very difficult to assemble by current synthetic methodology. We are interested in understanding the biosynthetic transformations utilized by Nature to stitch together such structurally and functionally exciting small molecules. By combining modern methods of Synthetic Biology, Molecular Biology and Biochemistry, we aim for a detailed mechanistic understanding of the underlying enzymatic processes. The resulting knowledge facilitates the incorporation of enzyme-catalyzed reactions in streamlined natural product total syntheses. Within this talk, our recent findings on strategies for the efficient cloning of small molecules biosynthetic pathways, the biosynthesis of potent aquatic toxins and on the chemo-enzymatic assembly of an entire class of complex fungal polyketides will be presented.

The evolution of genome mining for secondary/specialized metabolite biosynthetic gene clusters

Tilmann Weber

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Genome analyses of many microorganisms indicate that the genetic potential to synthesize specialized metabolites is far greater than the number of molecules observed in traditional screenings. Thus, genome mining for natural products biosynthetic pathways has emerged as an important technology complementing the chemical approaches.[1,2] In this pitch-talk, I will introduce the basic concepts and "evolution" of genome mining into a mature technology.

A key task in the whole genome mining workflow is the efficient identification of biosynthetic gene clusters (BGCs) in genomic data and the computational classification and (ideally) product prediction. In close collaboration with the group of M. Medema at Wageningen University, we are coordinating the development of the comprehensive genome mining platform antiSMASH (https://antismash.secondarymetabolites.org/)[3], which offers a one-stop solution for these key tasks. We recently released version 5 of antiSMASH1, including an improved user interface, new detection modules and many internal optimizations. With antiSMASH users can easily genomic sequences for the presence of secondary metabolite biosynthetic gene clusters. To provide extensive analysis options of the data generated with antiSMASH, we have added the antiSMASH database (https://antismash-db.secondarymetabolites.org/)[4] to the antiSMASH-framework, a user-friendly application allowing to browse and query antiSMASH annotation of >6,000 high-quality and >18,000 bacterial genomes.

These software tools build the basis for experimental studies such as cloning and heterologous expression or direct engineering of the "original" microbial producers.

References

1 Ziemert, N., Weber, T. & Medema, M. H. in *Comprehensive Natural Products III: Chemistry and Biology Reference Module in Chemistry, Molecular Sciences and Chemical Engineering* (ed Chaitan Khosla) DOI: 10.1016/b1978-1010-1012-409547-409542.414627-x (Elsevier, 2019).

2 Ziemert, N., Alanjary, M. & Weber, T. The evolution of genome mining in microbes – a review. *Nat. Prod. Rep.* **33**, 988-1005, doi:10.1039/c6np00025h (2016).

3 Blin, K. *et al.* antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res.* **47**, W81-W87, doi:10.1093/nar/gkz310 (2019).

4 Blin, K. *et al.* The antiSMASH database version 2: a comprehensive resource on secondary metabolite biosynthetic gene clusters. *Nucleic Acids Res.* **47**, D625-D630, doi:10.1093/nar/gky1060 (2019).

Marine biotech for biomedical applications: European Marine Biology Resource Center (EMBRC) Unlocking the potential of marine biotechnology

Marco Borra

Stazione Zoologica Anton Dohrn, Napoli, Italy

Marine biotechnology explores and uses marine bioresources as the target for origin of biotechnological applications, which are used for the production of products and services. Marine biodiversity is a rich source of medicines and natural products, potentially exploitable in the blue biotech industry. The marine environment is our ocean of opportunity for new materials, new compounds and new processes for our society. Advances in genomics and computer science have transformed earlier views of the ocean. It is no longer simply a source of food, but a vast reservoir of genetic potential and a means of achieving a wide range of socio-economic benefits. Genome sequencing is no longer the barrier it was a decade ago and our understanding of marine bioresources has improved significantly. However, new infrastructures are needed, with new models, new culture systems and new bioinformatics-based approaches to visualize genomics and other types of data. EMBRC-ERIC will be a global reference Research Infrastructure for fundamental and applied marine biology and ecology research, providing a single access point to a unique portfolio of services, resources and knowledge

ORAL PRESENTATION ABSTRACTS

Mining metagenomes of *Geodia barretti* from different depths for biosynthetic gene clusters

Asimenia Gavriilidou

Wageningen University & Reasearch, Wageningen, The Netherlands

The combat against cancer and the rise of antimicrobial resistance are among the greatest challenges of the 21st century. In order to address these global health issues, the discovery and development of new types of compounds with pharmaceutical properties have become top priority. Marine sponges are considered as the most prolific reservoir of pharmaceutically important natural products that appear to be mostly produced by the sponge-associated microorganisms. However, the majority of microbes are recalcitrant to cultivation under laboratory conditions while many gene clusters of previously cultured microbes remain cryptic, limiting accessibility in the real metabolic biodiversity. Aim of this study is to investigate the holobiont of a deep-sea sponge (Geodia barretti) of the North Atlantic, a known source of important secondary metabolites. Metagenome-mining revealed that sponges had higher numbers and more novel biosynthetic gene clusters (BGCs) compared to seawater. The secondary metabolite potential differed with depth as sponges from deeper seawater showed more novel BGCs and specifically more NRPS and PKS BGCs. Additionally, 154 high-quality metagenome-assembled genomes were retrieved from the deep-sea sponges. Further analysis will provide insights into the metabolic profile of the sponge-associated microbes and potential producers of bioactive compounds, facilitating the design of cultivation experiments. Mining the (meta)biome of an unexplored ecological niche such as deep-sea sponges, will enhance the chances of elucidating novel molecules.

Bioprospecting Marine Actinomycetes for Novel Anti-Tubercular Drugs

Daniela Tizabi

University of Maryland Center for Environmental Science

Mycobacterium tuberculosis, the pathogen behind the infectious lung disease Tuberculosis (TB), is estimated to currently infect 1.7 billion people worldwide. Although most of these cases are latent, the disease still poses a serious threat to individuals who are immunocompromised, such as those living with HIV/AIDS. This risk, coupled with the rise in antibiotic resistance, significantly hinders efforts set forth by the World Health Organization to end the epidemic by 2035. In the search for anti-TB drugs with novel modes of action, marine actinomycetes may serve as a promising source. Often found in symbiotic relationships with marine invertebrates, including sponges, these microbes produce a wide arsenal of compounds that aid in the defense of their non-motile hosts. Terrestrial actinomycetes are well known to produce secondary metabolites with pharmaceutical potential, yet those in the marine environment remain severely underexplored. A collection of novel marine actinomycetes previously isolated from a Caribbean giant barrel sponge, *Xestospongia muta*, was investigated for the potential to produce anti-TB compounds. Organic extracts were prepared from cultured strains that were then tested for growth-inhibition against various Mycobacterium species. Three isolates, pertaining to strains of Brevibacterium, Micrococcus, and Micromonospora, were shown to consistently inhibit Mycobacteria growth. Following these results, both a genomic and chemistry-enabled approach were carried out to determine the compound(s) responsible for the bioactivity. WGS was performed on the three strains; genomes were assembled using SPAdes, annotated using PATRIC, and potential biosynthetic gene clusters were identified using antiSMASH. However, it is still unclear which clusters possibly code for compounds effecting growth inhibition. Therefore, cross-referencing this data with chemical analysis by means of HPLC-MS and NMR is necessary to further isolate the compound(s) of interest. Preliminary MS analysis has thus far revealed an unknown compound of approximately 1200 Da in the active extract of the Micrococcus strain.

Nutrient starvation to enhance bioactivity in microalgae

Chiara Lauritano

Stazione Zoologica Anton Dohrn, Napoli, Italy

The EU project Pharmasea, ended in 2017, was a large consortium of 26 partners whose aim was to discover new bioactive compounds from marine microorganisms for the treatment of inflammatory, infective and neurodegenerative diseases. I will present some of the PharmaSea results on marine microalgae and recent data. Species that have shown anti-grazing or anti-proliferative activity on their principal predators, including crustacean copepods, were cultured in different culturing conditions in order to trigger the activation of metabolic pathways that can be silent in control condition. Previous studies have shown that many microalgal species produce defense metabolites with potentially interesting biotechnological applications when they are grown in stressful conditions such as nitrogen- and phosphate- starvation. Microalgal biomasses from 100 different species, grown in 3 culturing conditions (300 samples) were screened for different bioactivities (i.e. anti-inflammatory, anticancer anti-seizure and antimicrobial activities) and for active species RNA-seq was also performed. In particular, RNA-seq was performed for both the active and the inactive culturing condition, in order to identify differentially expressed enzymes potentially involved in the synthesis of the active metabolites.

Fungal cultivability and activation of silent gene clusters

Teppo Rama

UiT-The Arctic University of Norway

Major advances have been made during the past years of how microbes are cultured for natural products discovery. Most of the new culturing techniques aiming to trigger the growth of previously uncultured microbes with putatively novel natural products or activate silent gene clusters of cultivable microbes have been applied to bacteria. This presentation focuses on fungi and summarizes techniques tested to increase their cultivability and production of secondary metabolites in laboratory conditions.

POSTER ABSTRACTS

Heavy metal remediation from microalgae, potential of metallothioneins and phytochelatins

Sergio Balzano

Stazione Zoologica Anton Dohrn, Napoli, Italy

The ability of some eukaryotic microalgae to thrive in environments contaminated by heavy metals (HMs) is attracting the interest of the biotech industry for bioremediation purposes. Passive adsorption onto cell walls, active sequestration by metal-binding proteins, and compartmentalisation in vacuoles and other organelles are under investigation. Microalgae possessing cell wall polymers with negatively polar groups appear particularly suitable for HM adsorption. Free-living and immobilised microalgae can be used to remove HMs from sediment, and water, respectively. Furthermore, the rate at which HMs are transported across cell membranes and within cells can be improved by increasing the number and the efficiency of metal-binding cysteine-rich proteins such as metallothioneins (MTs) and phytochelatins (PCs). However most known MTs and PCs were identified from multicellular organisms. We searched within microalgal genomes and transcriptomes for the presence of novel genes coding MTs as well as the enzymes involved in PC-biosynthesis. We found 521 proteins, mostly from ciliates, dinoflagellates, diatoms, Cryptophyceae, Prymnesiophyceae, and Pelagophyceae, which possess features typical of MTs. In addition we found a number of genes potentially coding for the glutamate-cysteine ligase, the glutathione-synthase, and the phytochelatin synthase, the three enzymes involved in the biosynthesis of PCs. Genomic and transcriptomic investigation of microalgal communities from contaminated sites might contribute designing effective strategies for HM bioremediation.

Biosynthesis of long chain aliphatic compounds from *Nannochloropsis* spp

Martina Blasio

Stazione Zoologica Anton Dohrn, Napoli, Italy

The use of biological sources to produce sustainable materials has attracted great attention from the chemical industry since natural products can replace substances of petrochemical origin. In this direction, microalgae are promising because of their cost-effective growth and high lipid content, and the most suitable candidate species include *Nannochloropsis* representatives. In addition to the high content of C14-18 fatty acids and polyunsaturated fatty acids, *Nannochloropsis spp*. also produce long chain hydroxy fatty acids (LCHFAs) that, along with ordinary C14-18 fatty acids, can be used as substrates for methanol transesterification leading to the formation of fuels. Furthermore, LCHFAs from plants have been previously shown to improve fuel lubricity suggesting a similar role for *Nannochloropsis* LCHFAs. With respect to the sustainable production of materials, *Nannochloropsis spp*. produce two other classes of compounds structurally related to LCHFAs, the long chain diols (LCDs) and long chain alkenols (LCAs), which can be tested as a sustainable starters for the industrial synthesis of biopolymers. LCHFAs, LCDs and LCAs are likely to originate from the elongation and hydroxylation of C16-18 fatty acids, and polyketide synthase (PKS) and fatty acid elongases (FAE) have been suggested to be involved in this biosynthetic pathway. Specifically, PKS from *Nannochloropsis spp*. contain the active sites for ketosynthesis and ketoreduction, potentially catalysing an incomplete fatty acid elongation in which the C16:0 and C18:0 fatty acids would be elongated to form the 3-OH-C18:0/3-OH-C20:0 fatty acids. However, many intermediates of this hypothetical pathway have not been detected in *Nannochloropsis spp*., the role of PKS and FAE enzymes needs to be proven experimentally, and the enzymes catalysing the reduction of LCHFAs to LCDs are unknown. Intermediates are likely to be rapidly taken up by the enzymes catalysing the following reaction step and the use of crashed cellular material with active enzymes and the appropriate substrates might be a suitable approach to obtain detectable intermediates. Subsequently, we will confirm the activity of PKS and FAE enzymes by heterologous expression of their coding genes into yeasts and/or bacteria as well as by knocking down these genes in *Nannochloropsis spp.* Furthermore, these genes will be also overexpressed in *Nannochloropsis* strains to increase the production of LCHFAs. Finally, other culture manipulations coupled with transcriptome sequencing will be carried out in order to identify the enzymes involved in the reduction of LCHFAs to LCDs. The final goal of this project is to compare the production rates of LCAs, LCDs, and LCHFAs between the engineered yeasts and *Nannochloropsis* mutants in order to evaluate the most suitable technique for the industrial production of these lipids.

Novel biological target of Phomoxanthone A identified in Urea Cycle

Sara Ceccacci

Università degli Studi di Salerno, Salerno, Italy

Phomoxanthone A (PXA) is a tetrahydroxanthone dimer isolated from the endophytic fungus Phomopsis longicolla. Recent studies revealed that it disturbs the form and the function of inner mitochondrial membrane (IMM) in several ways. Indeed, PXA causes the rapid inhibition of the electron transport chain, the loss of the membrane potential ($\Delta\Psi m$), the release of mitochondrial Ca^{2+} and the fragmentation of the IMM. However, the identification of the mitochondrial interactome of PXA in an unbiased way has not been performed so far.

To determinate the targets of this natural compound, the chemoproteomic approach of Drug Affinity Responsive Target Stability (DARTS) has been performed on mitochondrial lysates. Obtained results have been validated by Western Blotting and LiP-MS, a method that couples limited proteolysis (LiP) with multiple reaction monitoring mass spectrometry (MRM-MS). DARTS relies on the proteolysis protection conferred on a protein by the interaction with a ligand: under physiological conditions, a protein fluctuates between multiple alternative conformations ("breathing"), but, upon saturation with a specific ligand, the equilibrium shifts to favor the thermodynamically more stable bound-ligand state, decreasing protein susceptibility to proteases degradation. LiP-MS enables to validate the small molecule interactome, shedding lights on the protein regions protected by PXA through the quantitation of its proteolytic peptides by means of MRM based strategies.

DARTS revealed Carbamoyl-phosphate synthase 1 (CPS1) as a novel biological target of PXA, confirmed by Western Blotting and LiP-MRM MS experiments. CPS1 plays an important role in removing excess ammonia from the cell, catalyzing the first reaction of the urea cycle. Activity assays in presence of PXA suggest a positive modulation of CPS1 activity by this compound.

Thus, PXA can be considered as a potential new lead compound for the treatment of CPS1 deficiency, a recessively inherited urea cycle error due to CPS1 gene mutations, which leads to life-threatening hyperammonemia.

A multiplex vector-based cell sensor for PPARs related drug discovery

Isabel Cunha

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Portugal

HPLC fractions of marine cyanobacterial extracts are screened with a vector system to bioprospect the LEGE culture collection (LEGE-CC; http://lege.ciimar.up.pt/), hosted at CIIMAR, for PPAR ligands. A multiplex cell sensor based on a transactivation assay, using two vectors, and two luciferases, allows for the detection of PPAR ligands. Three PPARs are identified at the time: □, □ and □. This assay was already in use for toxicological and phylogenetic studies and was upgraded to allow for high throughput screening. Upgrade concerned the luciferases used, the chemistry of the luciferase reactions, and the increase of information obtained per well. The vectors used are Promega modified vectors. The reporter vector (pGl4.35) was modified replacing the firefly luciferase (Fluc2P) by the high-intensity luminescence and stable NanoLuc® luciferase (NlucP), while the test vector [pFN24A (pBIND) hRluc-neo Flexi® vector], where the PPARs' ligand binding domain is expressed and a renilla luciferase (Rluc) was used for normalization purposes, the latter was replaced by the firefly luciferase (Fluc2), to increase the duration of the signal obtained. Moreover, in the pFN24A vector, the SV40 early promotor was replaced by the TK promoter to avoid excessive signal intensity due to the multiplex strategy. The kit used to perform the assay is the Nano-Glo Dual-Luciferase Reporter (DLR) Assay System (PROMEGA). The screening process is in progress and both PPAR agonist and antagonists are expected to be found among the cyanobacteria secondary metabolites. Marine cyanobacteria were chosen due to the greater richness of marine born organisms in polyunsaturated fatty acids (LCPUFA) as compared to freshwater, and the wealth of secondary metabolites of cyanobacteria. Natural ligands of PPARs are PUFA and ω3/ω6-PUFA oxidised derivatives, collectively termed oxylipins, biosynthesised mainly via cyclooxygenase, lipoxygenase, and cytochrome P450 pathways. However, plenty of novel oxylipin structures have been identified in marine organisms, produced through different biosynthetic pathways and featuring potent biological effects, which makes marine oxylipins promising targets for drug discovery. Oxylipins have been linked to several diseases including diabetes, dyslipidaemia, cardiovascular diseases, thrombosis, among others. Leukotrienes and prostaglandins are known to control inflammatory processes, allergic, and stress responses to infection and xenobiotics, in mammals.

“Omic” approaches for the identification of biosynthetic pathways of bioactive compounds from marine microalgae

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Microalgae are a group of eukaryotic and unicellular microorganisms with a great morphological and metabolic diversity. Among them, diatoms are of particular importance in the biogeochemical cycles of many nutrients and are responsible of the great part of the primary productivity in the oceans (1). Diatoms live in a very challenging environment, and for this reason they possess a huge molecular toolkit, not fully studied, promising source for the “Blue Biotechnology”. The Blue Biotech is a rising field of study that use marine organisms and marine environment as sources of new bioactive compounds useful for biotechnological, pharmaceutical and industrial purposes (2). A centric diatom species, Thalassiosira rotula, from the Gulf of Naples, has been demonstrated be able to synthesize very interesting bioactive molecules called oxylipins, produced after cell disruption, able to impair reproduction of organisms that feed on this species (3). More recently, using an omic approach, in this specie has been detected also the expression of the metabolic pathways for prostaglandins, secologanin and polyketides (4). This is just an example of the omic approach potential to discover new molecules with anti-microbial, antitumoral and anti-inflammatory activities by the simultaneous analysis of a large amount of data generated by the genome, transcriptome and proteome sequencing.

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Enhancing bioactive compounds in the marine diatom *Phaeodactylum tricornutum*.

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Diatoms are a major group of microalgae found in all aquatic ecosystems, adapted to different and sometimes extreme environments. They naturally produce various substances beneficial for human health, food and feed, such as polyunsaturated fatty acids, vitamins, antioxidants, enzymes, polysaccharides and carotenoids, and therefore represent a potential source for commercial and industrial applications. Our research focuses on the improvement of phytotherapic compounds in the marine diatom Phaeodactylum tricornutum. This is a very promising microorganism for commercial and industrial application processes, its growth is rapid (biomass doubling in a few hours), cost-effective, and sustainable since it can be easily controlled in outdoor and indoor conditions. To facilitate exploitation of this diatom several resources are available, including genome, transcriptomes and tools for genome editing. We focused our work on carotenoids and xanthophylls, known for their anti-oxidant activity, anti-metabolic syndrome activities (anti-obesity, anti-diabetes) and beauty-enhancing activities (skin-enhancing, skin-lightening, anti-acne). In order to increase synthesis of these pigments in Phaeodactylum tricornutum, we exploited genetic engineering techniques to overexpress simultaneously different genes involved in the carotenoid and xanthophylls biosynthetic pathway obtaining multiple genes transgenic lines with increased carotenoid content.

Heterologous production of a microalgal putative protein pheromone

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The increasing number of genomic projects concerning marine organisms is leading to the identification of a growing number of genes coding for proteins with a putative biotechnological interest. Based on sequence analysis, a large part of the genes identified from these genomic data have unknown cellular and/or molecular functions. To understand the precise biological function of these gene products, their biochemical and physiological characterization is essential and, due to the complicated purification process from the natural organisms, heterologous expression systems would be very useful. To this aim, we would like to establish at the SZN a medium-throughput strategy to produce large amounts of marine-derived soluble proteins, testing different E. coli strains as host bacterium and different expression vectors under different growth conditions to find the optimal production strategy. As pilot project, we choose to produce MRP1, a protein coded by a gene overexpressed during the early phase of the sexual reproduction in the diatom *Pseudo-nitzschia multistriata*. Diatom sex is a subtle event with a heavy impact on ecosystem dynamics. Understanding the mechanisms that regulate the onset of diatom sex in nature is important for a better comprehension of phytoplankton life cycles, which impact on population dynamics. MRP1 is predicted to contain a putative signal peptide that targets proteins for translocation across the endoplasmic reticulum (ER) membrane, suggesting that the protein might be secreted. Hence, the strong upregulation of this gene during sexual reproduction and the presence of a putative signal peptide suggest that MRP1 may play a role as sexual pheromone. Pheromones comprise a wide range of compounds, of varied chemical nature, that range from small molecule volatile compounds to high-molecular-weight, water-soluble, nonvolatile peptides and proteins; in particular, there are several examples of pheromones of peptidic nature in algae. To test the role of MRP1 as signaling molecule, we produced the MRP1 protein in a heterologous system. To this aim, we adopted the strategy developed by the Oxford Protein Production Facility(OPPF); combining the easy cloning system of the In-Fusion™ enzyme to a set of pOPIN vectors, we generated different expression vectors in which MRP1 was fused to different tags. Each of these vectors was transformed in several distinct E. coli strains (optimized for heterologous recombinant proteins production) and their expression was induced at different temperatures. The best setup for the protein production was determined analyzing the cells extracts for each strain/growth condition. The purified protein will be tested on *P. multistriata* cell cultures. In particular, female strains will be exposed to different concentrations of the protein to verify if this acts as a male pheromone, stimulating changes in the expression levels of genes found to be differentially expressed in females during mating. In conclusion, this project provides purified proteins of marine origin that are key reagents for numerous assays that address fundamental questions about their structure, function and regulation.

Exploiting sponge-microbe interactions for the discovery of novel natural products

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Abstract Body Text: Sponges (Phylum Porifera) are ancient invertebrate metazoans, with a fossil record dating back to 600 million years that host complex and diverse microbial communities such as archaea, cyanobacteria, heterotrophic bacteria, algae, fungi, and dinoflagellates. In the marine environment sponges and/or associated microbes are considered as the chemical factory because of its large production of chemically diverse natural products with remarkable bioactivities. The large spectrum of these bioactivities include anticancer, antiviral, antibacterial, antifungal, antiprotozoal, anthelmintic, anti-inflammatory, immunosuppressive, neurosuppressive, neuroprotective and antifouling, among others.

Sponge-associated microbes and their bioactive compounds have been studied in samples from wide oceans across the globe, mainly in subtidal regions, but remain mostly unexplored in intertidal sponges. Till date, it was only possible to unlock a fraction of the microbial consortium present in the sponge host.

In order to explore the ecological and evolutionary nature of the symbionts, using advanced sequencing technologies; sampling campaigns of distinct intertidal marine sponge species were conducted in rocky beaches along the Portuguese coast. A metabarcoding approach was designed to unlock the microbial consortium present in the sponge host. In order to unravel the biotechnological potential of the collected species we intend to: (1) perform metagenomics studies to search for genes responsible for secondary metabolites production, and (2) assess the metabolic diversity to identify sponge products with bioactive potential. It is expected that the combination of these techniques with bioactivity-guided isolation will lead us to discovery of novel and bioactive natural products.

Disclosing the key biological target of Crellastatin A through a combination of proteomic approaches.

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The identification of target proteins of natural products is crucial to understand their mechanism of action in order to develop new molecular probes and/or therapeutic drugs.

In this scenario, we applied the Drug Affinity Responsive Target Stability (DARTS) approach to characterize the interactome of Crellastatin A (CreA), a marine sulphated bis-steroid from the sponge Crella sp. [1].

This strategy relies on the evidence that a protein might become less susceptible to proteolysis when it is bound to a molecule, as a result in the shift of its thermodynamic landscape to favor the more stable ligand-bound state [2]. The conformational changes between the protein and its ligand-bound form can be detected by SDS-PAGE and mass spectrometry, through the altered proteolytic pattern reached by the protein when exposed to an unspecific protease, such as subtilisin. DARTS led to the identification of Poly [ADP-ribose] polymerase 1 (PARP-1) as CreA most reliable partner, as also validated by immunoblotting.

In order to identify PARP1 regions involved in the interaction with CreA, we also performed LiP-MRM (Limited Proteolysis-Multiple Reaction Monitoring) experiments [3].

Because of the natural compound exerted protection, PARP1 region(s) interacting with CreA are less prone to subtilisin digestion and thus generate more abundant fully tryptic peptides compared to a control sample, which is in turn more prone to subtilisin and will produce more half-tryptic peptides. Tryptic peptides intensities can be easily measured by MRM-MS directly in a complex proteome matrix, without sample enrichment or protein labeling. LiP MRM obtained data were corroborated by blind molecular docking, pointing out PARP-1 WGR domain as a putative CreA/PARP-1 binding site. Finally CreA inhibitory activity on PARP-1 has been assessed through an in vitro assay carried out on the human recombinant PARP1.

Microalgal Enzymes with Biotechnological Applications

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Enzymes are essential components of biological reactions and play important roles in thescaling and optimization of many industrial processes. Due to the growing commercialdemand for new and more efficient enzymes to help further optimize these processes,many studies are now focusing their attention on more renewable and environmentallysustainable sources for the production of these enzymes. Microalgae are very promisingfrom this perspective since they can be cultivated in photobioreactors, allowing theproduction of high biomass levels in a cost-efficient manner. This is reflected in theincreased number of publications in this area, especially in the use of microalgae as a sourceof novel enzymes.In particular, various microalgal enzymes with different industrial applications (e.g., lipidsand biofuel production, healthcare, and bioremediation) have been studied to date, and themodification of enzymatic sequences involved in lipid and carotenoid production hasresulted in promising results. However, the entire biosynthetic pathways/systems leading tothe synthesis of potentially important bioactive compounds have in many cases yet to befully characterized (e.g., for the synthesis of polyketides). Nonetheless, with recent advancesin microalgal genomics and transcriptomic approaches, it is becoming easier to identifysequences encoding targeted enzymes, increasing the likelihood of the identification,heterologous expression, and characterization of these enzymes of interest.My PhD thesis research focuses on the analysis of marine microalgal transcriptomes,previously sequenced during the EU-FP7 PharmaSea project or newly sequenced, in order toidentify sequences encoding for enzymes involved in biosynthetic pathways ofbiotechnological interest. The most promising sequences will be expressed in heterologoussystems and the produced protein will be further studied via activity assays and structuralcharacterization, also evaluating the marketability of the produced enzyme.