

Effect of falcarindiol and falcarinol purified from carrots on inflammation and cancer

Arnsfang, Eva Christensen; Pipó-Ollé, Emma ; Notabi, Martine K.; Hedegaard, Martin A.B.; Walther, Anders; Kobaek-Larsen, Morten; Baatrup, Gunnar; El-Houri, Rime Bahij; Christensen, Lars Porskjær

Published in:

Nordic Natural Product Conference (NNPC) 2019, Iceland

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Arnsfang, E. C., Pipó-Ollé, E., Notabi, M. K., Hedegaard, M. A. B., Walther, A., Kobaek-Larsen, M., Baatrup, G., El-Houri, R. B., & Christensen, L. P. (2019). Effect of falcarindiol and falcarinol purified from carrots on inflammation and cancer. In *Nordic Natural Product Conference (NNPC) 2019, Iceland* (pp. L26, p. 15)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

NNPC CONGRESS **ABSTRACT BOOK**

2019

JUNE 9–12,
FLÚÐIR, ICELAND



NORDIC NATURAL
PRODUCT CONFERENCE

Invited lectures

IL01

Integrated Metabolomics and Mass Spectrometry Imaging In Marine Drug Discovery and Chemical Ecology

Tasdemir Deniz

GEOMAR Helmholtz Centre for Ocean Research Kiel, KIEL, Germany

Marine natural products (MNP) research is experiencing a paradigm shift towards microbes due to recognition of the holobiont concept, plus the demonstrated/predicted microbial origin of many bioactive MNPs. However only 1% of the microbial diversity is cultivable in laboratory conditions leading to the 'microbial dark matter' in which large portions of biosynthetic gene clusters remain dormant. This renders chemical capacity of marine microbes inaccessible. Other challenges involve the very low (<2%) annotation rate of MNPs by classical metabolomics approaches and lack of insights into roles of MNPs in host-microbe or microbe-microbe interactions that underlie their pharmacological activities. All these major obstacles require innovative and high throughput methods. In order to address microbiological dark matter and to enhance cultivability and chemodiversity of marine microorganisms, we apply OSMAC, co-cultivation and microchip techniques. The remaining dark matters are dealt with LC-HRMS/MS based molecular networks and other automated metabolomics tools that significantly increase annotation rates of MNPs and allow prediction/mapping the bioactivity to molecular networks. Imaging mass spectrometry (IMS) extends the power of MS by providing the chemical/spatial information on marine surfaces. This presentation will summarize our integrated research combining massive metabolomics, chemical imaging, microbiome and traditional bioassays in marine biodiscovery and chemical ecology.

IL02

Is there a future for natural products as anti-infective agents?

Paul Cos

University of Antwerp, ANTWERP, Belgium

There is still a great interest in plant-derived compounds as potential antibacterial agents (1). The compounds can act in three different ways, i.e. direct antibacterial activity, anti-virulence or resistance-modifying activities. During the last few decades most research efforts have focused on finding novel antibiotics from plants. The outcome was very disappointing, especially for compounds with an activity against Gram-negative bacteria. The more promising strategies for plant-derived compounds are those that study their anti-virulence and resistance-modifying activities. This paradigm shift is now ongoing, but several hurdles may hamper its success. First, more complex and expensive bioassays are required for this type of research. Second, the correct interpretation of the results is difficult and prone to artefacts. Third, one should look for combination therapies with existing antibiotics, again complicating the set-up and interpretation of experiments. Nevertheless, recent publications are very promising and should encourage more researchers to study natural products as potential anti-infective agents.

(1) Bidart de Macedo Maira et al., Anti-infective agents: the example of antibacterial drug leads, *Ethnopharmacology* / Heinrich, Michael [edit.]; et al. - ISBN 978-1-118-93074-8 - Malden, Mass., Wiley-Blackwell, 2015, p. 111-121

Reference :

Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B2610 Antwerp, Belgium, paul.cos@uantwerpen.be

IL03

Induced expression of antimicrobial peptides to fight infections

Guðmundur Hrafn Guðmundsson

University of Iceland, REYKJAVIK, Iceland

Induced expression of human antimicrobial peptides has been the emphasis of our research. This approach could be an alternative to antibiotic treatment. Background of the basic concept will be covered in the talk. Possible usage for the induction of innate immunity against selected infections in animal models will be discussed as host directed therapy (HDT). A cell model for selecting novel inducers of innate immunity has been used. The discovery of the most recent novel inducers and their activity with respect to regulation of innate immunity, affected gene expression, specific epithelial responses and possible molecular mechanism will be presented in details.

Session 1

L01

Molecular networking as Tool for discovering anti-parasitic compounds from Seaweed

Henrik Toft Simonsen¹, Louis Bornancin¹, Charlotte Smith Bonde², Andrew Richard Williams², Helena Mejer², Stig Milan Thamsborg²

¹Technical University of Denmark, KONGENS LYNGBY, Denmark

²Faculty of Health and Medical Sciences, University of Copenhagen, FREDERIKSBERG, Denmark

Seaweeds contains many bioactive compounds with activities towards bacteria, parasites, and fungi. We have combined molecular networking using GNPS (Global Natural Product Social molecular networking) and bioguided-fractionation to find anti-parasitic compounds in four Nordic seaweeds species: *Saccharina latissima*, *Laminaria digitata*, *Ascophyllum nodosum*, and *Palmaria palmata*. The *in vitro* anthelmintic activity was assessed using an *Ascaris suum* (the large roundworm of pig) third stage larvae (L3) in a mortality assay (1 mg/mL was dissolved in DMSO)[1]. Lipophilic extracts (hexane and dichloromethane:methanol extracts) showed higher activity than the polar extracts (water and methanol). The most potent extracts originated from *S. latissima* and *L. digitata*, with an average mortality of >95% after 48 hours. Bioguided-fractionation GNPS molecular networking enabled us to identify bioactive fractions from *S. latissima* (94% mortality, 0,5mg/mL) and *L. digitata* (87% mortality, 0,5mg/mL) containing free fatty acids. The purified compounds only had moderate mortality (15 to 64% at 50µg/mL), highlighting that the observed activity arise from a synergic effect of the compounds with antiparasitic activity.

We conclude that the Nordic seaweeds *Saccharina latissima* and *Laminaria digitata* have *in vitro* AH effects against common pig and sheep nematodes, and the AH activity is caused mainly by free fatty acids.

Reference :

[1] Peña-Espinoza, M; Valente, A.H.; Thamsborg, S. M.; Simonsen, H. T.; Boas, U.; Enemark, H. L.; López-Muñoz, R.; Williams, A. R. *Parasites & Vectors* **2018** *11*, 475.

L02

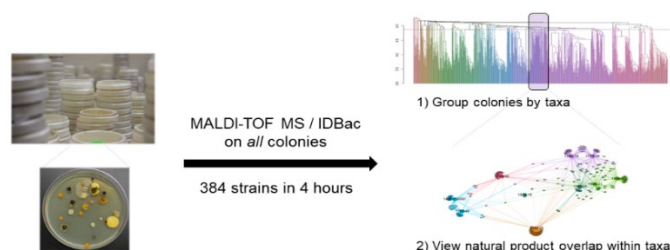
Creation of Diverse Microbial Libraries for Drug Discovery using MALDI-TOF MS / IDBac

Maria Costa¹, Chase Clark², Sesselja Omarsdottir¹, Laura Sanchez², Brian Murphy²

¹University of Iceland, REYKJAVIK, Iceland

²University of Illinois at Chicago, CHICAGO, United States of America

In the course of a nearly century-long global effort to discover new bacterial-derived antibiotics from the environment, there have been few innovations to the way that researchers have collected samples and subsequently created microbial libraries sourced for therapeutic discovery. Virtually every stage of the natural products (NP) drug discovery process has undergone a renaissance in method innovation: analytical detection of NPs, bioinformatic-driven discovery of NPs, detection, modification and expression of NP biosynthetic gene clusters, structure elucidation and dereplication of NPs and NP biological activity screening. Despite these advances, the philosophy we use to create microbial strain libraries from the environment has seen little change in nearly seven decades of biomedical research. As a result, it is difficult to discover novel antibiotic scaffolds due to the degree of taxonomic and chemical redundancy that exists in these strain libraries, which led to a divestment in microbial-based natural product drug discovery. To address the need for isolating novel and/or chemically diverse taxa from environmental samples, we developed a high-throughput matrix assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) technique that allows us to readily group bacterial colonies by putative taxonomic identity and further discriminate them based on *in situ* natural product production.



Abstract figure: Creation of microbial libraries

Reference :

Clark, Chase M.; Costa, M. S.; Sanchez, L. M.; Murphy, B. T. Proceedings of the National Academy of Sciences **2018**, *115*, 4981.

Session 2

L03

Chemistry of fish killing microalgal toxins in genus *Prymnesium*

Thomas Ostenfeld Larsen

Technical University of Denmark, KGS. LYNGBY, Denmark

Species in genus *Prymnesium* are haptophyte microalga that form blooms leading to devastating fish kills worldwide. The most famous species within this genus is by far *P. parvum* known for production of ladder frame polyethers called prymnesins. Today, the structures of A- and B- type prymnesins have been characterized like MS/MS analysis indicates the presence of a C-type. In order to investigate the chemodiversity within *P. parvum* we screened 26 strains of *P. parvum* with a wide geographical distribution for their production of prymnesins. To our surprise this showed that the diversity of prymnesins is much larger than previously thought, since we could tentatively describe 51 different molecular features (9 A-type, 12 B-type and 30 C-type prymnesins). Furthermore, we performed phylogenetic analyses based on internal transcribed spacer (ITS) sequences, both ITS-1 and ITS-2. A monophyletic origin of all types of prymnesins was revealed and clades could be defined by their type of toxic compound produced. This suggests that evolution of new species within the *P. parvum* species complex could be driven by changes in toxin type. Chemotaxonomy and ITS-type classification may thus be used to further delimit the *P. parvum* species complex.

Reference :

Binzer, S.B., Svenssen, D.K., Daugbjerg, N., Alves-de-Souza, C., Pinto, E., Hansen, P.J., Larsen, T.O., Varga, E. A-, B- and C-type prymnesins are clade specific compounds and chemotaxonomic markers in *Prymnesium parvum*. *Harmful Algae*, 2019, **81**, 10-17. doi.org/10.1016/j.hal.2018.11.010

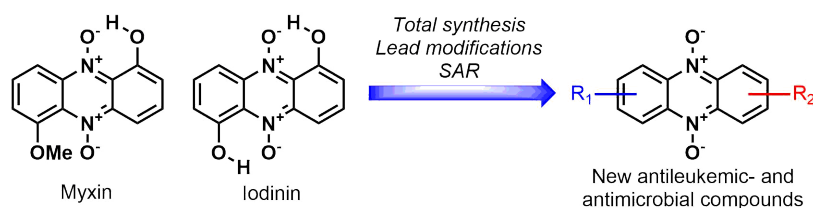
L04

Synthesis and biological evaluations of new phenazine 5,10-dioxides with antileukemic and antimicrobial activities derived from the natural products myxin and iodinin

Elvar Örn Viktorsson

University of Iceland, REYKJAVIK, Iceland

Heterocyclic aromatic *N*-oxides are of rare occurrence in nature although they often display potent biological effects.¹ The phenazine 5,10-dioxide natural products myxin and iodinin are well known examples (Fig. 1).² Recent efforts have revealed iodinin to be a potent selective apoptotic inducer of leukemic cell death.^{3,4} Myxin on the other hand is a potent broad-spectrum antimicrobial agent.⁵ The presented work will cover the synthesis, biological evaluations and SAR results of new phenazine 5,10-dioxides aimed towards selective targeting of acute myeloid leukemia. Preliminary antimicrobial evaluations will also be discussed.



Abstract figure: Preparation of new analogs of the natural products iodinin and myxin for SAR studies in various cell lines.

Reference :

Org. Lett., 2016, **18**, 2495.
Annu. Rev. Phytopathol., 2006, **44**, 417.
Antimicrob. Agents. Ch., 1970, **10**, 46.
Bioorg. Med. Chem., 2017, **25**, 2285.
Mar. drugs, 2013, **11**, 332.

L05

Production of antimicrobial compounds by marine bacteria

Arnheidur Eythorsdottir¹, Sesselja S. Omarsdottir², Hjorleifur Einarsson¹

¹University of Akureyri, AKUREYRI, Iceland

²University of Iceland, REYKJAVIK, Iceland

Background: The search for novel antimicrobial compounds is important in many aspects such as for preservatives and possible drug leads, where drug resistance of pathogens is becoming a serious problem. New records of antimicrobial activity from marine bacteria emerge every year¹. The aim of this study was to search for bacterial antimicrobial compounds and study the conditions for their production.

Methods: Bacterial isolates were retrieved from invertebrates in the surroundings of underwater hydrothermal vents. Isolates were tested for antimicrobial activity by growing specific test strains in their presence. A few isolates were tested every 2 weeks over a 10 weeks period to find out the influence of culturing time on the activity. Isolates were identified by 16S sequencing.

Findings: Antimicrobial activity of marine actinomycetes and bacilli from the hydrothermal vent site was observed. A prolonged incubation time enhanced the effect on the present test strains.

Conclusion: The ecosystem at hydrothermal vent site is moderately rich of micro-organisms capable of producing antimicrobial compounds. On an agar medium, the production seems to go on independently of the presence of test strains. Isolation and identification of the antimicrobial compounds will shed more light on the subject.

Reference :

Mayer, Alejandro M. S.; Rodriguez, Abimael D.; Tagliatala-Scafati, Orazio and Fusetani N. 15 (9)

Session 3

L06

High-resolution bioactivity profiling combined with LC-HRMS/NMR analysis reveals anti-hyperglycemic and antibacterial diterpenes in Australian Eremophila species

Dan Staerk¹, Yong Zhao¹, Susan Semple²

¹University of Copenhagen, COPENHAGEN, Denmark

²University of South Australia, ADELAIDE, Australia

Background Type 2 diabetes affects around 425 million people worldwide [1], and is a chronic multifactorial disease characterized by hyperglycemia. Similarly, the increase in infections caused by various multidrug-resistant bacteria is also a major public health concern, with an estimated 700.000 deaths per year [2]. New chemical entities are therefore urgently needed for the management of these diseases.

Methods Polypharmacological high-resolution bioactivity profiling [3] of crude plant extracts allowed direct correlation of HPLC peaks with one or more bioactivities, and LC-HRMS/NMR were subsequently used to target structural identification of the active constituents.

Findings High-resolution inhibition profiling led to identification of 31 new serrulatane and viscidane type diterpenoids from *Eremophila rugosa* and *E. denticulate*, and five new furanosesquiterpenes from *E. bignoniiflora* - all associated with α -glucosidase and/or PTP1B inhibitory activity. The isolated compounds were also associated with antibacterial activity against one or more bacterial strains (MIC from 25 to 1600 mg/mL). Checkerboard assays revealed that one of the serrulatanes possessed a synergistic effect with oxacillin (FIC index 0.375-0.5).

Conclusion Polypharmacological bioactivity profiling of individual constituents in crude plant extracts disclosed a hitherto unexplored chemical diversity of anti-diabetic and antibacterial terpenes in *Eremophila* spp.

Reference :

[1] International Diabetes Federation. IDF Diabetes Atlas, 8th edition, **2017**. ISBN: 978-2-930229-87-4. <http://www.diabetesatlas.org/> Accessed 2019-02-26.

[2] O'Neill, J. 2016 Tackling drug-resistant infections globally: Final report and recommendations. The Review on Antimicrobial Resistance. <https://amr-review.org/> Accessed 2019-02-26

[3] Zhao, Y.; Kongstad, K.T.; Jäger, A.K.; Nielsen, J.; Staerk, D. Quadruple high-resolution α -glucosidase/ α -amylase/PTP1B/radical scavenging profiling combined with high-performance liquid chromatography - high-resolution mass spectrometry - solid-phase extraction - nuclear magnetic resonance spectroscopy for identification of antidiabetic constituents in crude root bark of *Morus alba* L. J. Chromatogr. A. **2018**, 1556, 55-63.

L07

Discovering valuable lipids of mosses from Iceland by UPLC-ESI-QTOF-MS based lipidomic approach

Yi Lu¹, Finnur Freyr Eiriksson², Margrét Thorsteinsdóttir², Henrik Toft Simonsen³

¹ArcticMass & Dechnical University of Denmark, REYKJAVÍK, Iceland

²ArcticMass, REYKJAVÍK, Iceland

³Technical University of Denmark, LYNGBY, Denmark

Bryophytes, mosses, liverworts and hornworts, produce a large variety of lipids such as terpenoids, sterols and fatty acids. Bryophytes produce relatively high amounts of very long-chain polyunsaturated fatty acids (VLC-PUFAs) including arachidonic acid (AA) and eicosapentaenoic acid (EPA), which are uncommon in higher plants. Such high contents of VLC-PUFAs highlight their potential usage in pharmaceutical industry, food industry and cosmetics. Icelandic mosses are adapted for the harsh environment with low temperature, which are expected to produce high contents of these valuable VLC-PUFAs.

The aim of this study is to discover fatty acids and their linked lipid classes in bryophytes species collected in Iceland by performing untargeted lipid profiling using ultra performance liquid chromatography coupled to electrospray ionization quadrupole-time-of-flight-mass spectrometry (UPLC-ESI-QTOF-MS). After comparing the total lipid content with different extraction parameters, including cell disruption (shake, ultrasound and bead beating) and sample condition (air-dried, freeze dried and fresh frozen), we concluded that using fresh frozen sample with bead beating assistant resulted the highest lipid yield. Future works will examine the lipid species coverage and reproducibility in order to choose a most suitable extraction method for further study.

L08

Application of Chitin derivatives - challenges in characterization and analysis of Chitin derivatives.

Pórlleifsdóttir Sigrún

Genís, REYKJAVÍK, Iceland

Chitin is an abundant biopolymer in nature, found mainly in the shell of crustaceans, consisting of N-acetyl-D-glucosamine units.

Chitin and chitin derivatives have been a topic of interest in the science community for several years and several application possibilities investigated in the food industry, agriculture and medicine.

It has been shown that the differing characteristics that the chitin derivatives possess affect the mode of action and give rise to the multiple specialized application possibilities.

Chitin derivatives exhibit widely differing physicochemical properties depending on the chitin source and the conditions of chitosan production and the characterization of the resulting derivative is often lacking.

The characteristics most investigated are the molecular weight and degree of acetylation/deacetylation but also of interest are the pattern of acetylation and polydispersity.

The analysis of these characteristics can be challenging due to the lack of standard methods and requirements and several papers have in fact been published addressing these challenges.

L09

Structure-based dual-target lead discovery against acetylcholinesterase and the $\alpha 7$ nicotinic acetylcholine receptor: From in silico studies to in vitro confirmation.

Sebastian Oddsson¹, Natalia Magdalena Kowal¹, Philip Kiær Ahring², Mary Chebib², Elín Soffía Ólafsdóttir¹, Thomas Balle²

¹University of Iceland, REYKJAVÍK, Iceland

²University of Sydney, SYDNEY, Australia

Despite extensive efforts in the development of drugs for complex neurodegenerative diseases, treatment often remains challenging or ineffective and hence the application of new strategies is necessary^{1,2,3}. One approach is the design of multi-target drugs, which can potentially address the complex nature of disorders such as Alzheimer's disease^{4,5}. We report the identification of a novel lead compound (Ymir-6) with dual-activity as an acetylcholinesterase (AChE) antagonist and as an $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) agonist using computational chemistry methods and subsequent *in vitro* validation by Ellman's method and two-electrode voltage-clamp electrophysiology, respectively. Parallel and independent screening of a virtual compound library, consisting of approximately 5,213,046 drug-like and commercially available molecules from the ZINC database, against the two protein targets resulted in an intersecting set of 57 potential dual activity compounds. Based on ligand efficiency, scaffold and molecular diversity, 15 of these compounds were purchased for *in vitro* testing. Ymir-6 was shown to exhibit the desired activity profile (AChE IC₅₀ = 1.8 μ M; $\alpha 7$ nAChR activation = 7.3 $\pm$ 0.9%) making it the first reported compound with this particular profile and providing further evidence of the feasibility of *in silico* methods for the identification of novel multi-target lead molecules^{6,7}.

Reference :

Youdim, M. B.; Buccafusco, J. J. *Trends in pharmacological sciences* **2005**, 26, 27

Millan, M. J. *Neurotherapeutics* **2009**, 6, 53

Bolognesi, M. L. *Current Medicinal Chemistry* **2013**, 20, 1639

Reddy, A. S.; Zhang, S. *Expert Review of Clinical Pharmacology* **2013**, 6, 41
 Hughes, R. E.; Nikolic, K.; Ramsay, R. R. *Frontiers in neuroscience* **2016**, 10, 177
 Kowal, N. M.; Indurthi, D. C.; Ahring, P. K.; Chebib, M.; Ólafsdóttir, E. S. *Molecules* **2019**, 24, 446
 Ramsay, R. R.; Popovic-Nikolic, M. R.; Nikolic, K.; Uliassi, E.; Bolognesi, M. L. *Clinical and translational medicine* **2018**, 7, 3

Session 4

L10

Using herbarium collections as a source of comparative chemical data over time and space

Nina Rønsted
 Natural History Museum of Denmark, COPENHAGEN, Denmark

Herbarium samples are a unique research structure presenting records in time and place, which can be used for studying distribution patterns of different traits such as occurrence data of rare taxa, invasive weeds and plant pathogens. Changes in flowering times, herbivory, or composition of a flora can also be studied. Although destructive sampling is generally not recommended from type specimens and more valuable collections, many species are represented by multiple collections, and smaller samples can be used for DNA studies or chemical studies. Herbarium specimens are often considered unsuitable for chemical studies because of challenges with expected degradation of chemical metabolites potentially rendering chemical data uncomparable across specimens or to modern samples. In this presentation, examples will be given of possible applications. A case study of volatile terpenoid profiles using 150 years of herbarium collections of four medicinal species of *Salvia* across the Mediterranean show strong species effect despite lower amounts compared to modern samples. Another case study using herbarium moss samples for biomonitoring of atmospheric deposition of persistent organic pollutants (POPs) in Greenland show clear changes across the 20th century, primarily related to changing industry.



Abstract figure: *Salvia* species for chemical studies using herbarium specimens

Reference :

Jafari Foutami I, Mariager T, Rinnan R, Barnes CJ, Rønsted N. Hundred fifty years of herbarium collections provide a reliable resource of plant metabolite data showing strong species effect in medicinal species of *Salvia* across the Mediterra

L11

Structural polysaccharide composition of Aloe species correlates with growth habit rather than phylogeny or medicinal use

Louise Isager Ahl¹, Christopher Barnes¹, Henriette L. Pedersen¹, Bodil Jørgensen², William G. T. Willats³, Olwen M. Grace⁴, Nina Rønsted¹

¹Natural History Museum of Denmark, COPENHAGEN, Denmark

²University of Copenhagen, COPENHAGEN, Denmark

³University of Newcastle, NEWCASTLE, United Kingdom

⁴Royal Botanic Gardens, Kew, RICHMOND, United Kingdom

Aloe vera inner leaf mesophyll (*Aloe* gel) supports a large global industry¹. The genus *Aloe* contains over 500 species with approximately 25% being used medicinally and it is unclear why only a few species of *Aloe* dominate the global market². The bioactive polysaccharides of *Aloe vera* gel have been extensively studied and the main bioactive component is thought to be an acetylated glucomannan. Genus-wide studies of polysaccharide compositions have been a missing link in understanding what determines this composition and whether the global market dominance of *Aloe vera* is correlated with a distinct polysaccharide profile.

We used the high-throughput capacity of Carbohydrate Microarray Polymer Profiling³ to analyse 94 species representing the diversity of aloes. The presence of six major groups of polysaccharides were targeted using 27 different monoclonal antibodies. Multivariate linear modelling of geographic region, biome, altitude, medicinal use, and habit, showed habit to be the predominant factor in predicting polysaccharide composition. A correlation with habit may be explained by the function of polysaccharides in cell wall structure. There was no correlation between *Aloe* phylogeny and their polysaccharide profiles, while it was not possible to differentiate the profiles of *Aloe vera* and other commercial species from non-commercially used species.

Reference :

1. Grace, O. M. Current perspectives on the economic botany of the genus *Aloe* L. (Xanthorrhoeaceae). *South African J. Bot.* **77**, 980–987 (2011).
2. Grace, O. M. *et al.* Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of *Aloe vera*. *BMC Evol Biol* **15**, 29 (2015).
3. Ahl, L. I. *et al.* Analyses of *Aloe* polysaccharides using carbohydrate microarray profiling. *J. AOAC Int.* **101**, 1711–1719 (2018).

L12

Ethnobotany of Iceland Moss (*Cetraria islandica*): DNA barcoding, chemotaxonomy, phylogeography and future perspectives

Maonian Xu¹, Starri Heidmarsson², Sesselja Omarsdottir¹, Elin Olafsdottir¹

¹University of Iceland, REYKJAVIK, Iceland

²Icelandic Institute of Natural History, AKUREYRI, Iceland

Iceland Moss is a lichen taxon named after its major lichen-forming fungus *Cetraria islandica*. It has been prepared as herbal tea and tincture, and used as a traditional medicine for the treatment of oral or pharyngeal irritation and associated dry cough. In Iceland, it is also used as a food ingredient in milk soup and bread, etc. However, the identification of *C. islandica* remains a problem, in terms of its high morphological and chemical variations and similarity to its sister lichen taxon *C. ericetorum*. They were collectively called *C. islandica* complex. The current study aimed to explore the use of DNA barcoding and chemical profiling to identify *C. islandica*, and to investigate the correlation between chemotypes and geographic distribution. In DNA barcoding analysis, the RPB2 locus outperformed the widely used fungal nrITS barcode. Chemical profiling combined with multivariate data analysis could recognize two *C. islandica* chemotypes and *C. ericetorum*. Phylogenetic analysis of over 160 specimens suggests that chemotypes show certain evolutionary independence, where fumarprotocetraric acid (FA)-deficient chemotype is only distributed in north Iceland, and the FA-producing one grows all around Iceland. On-going and future studies on Icelandic *C. islandica* will also be introduced, including fungal and algal cultivation, mycotoxin production.

Reference :

- Kristinsson, H. *The Bryologist* **1969**, 72, 344.
- Xu, M; Heidmarsson, S; Thorsteinsdottir, M; Kreuzer, M; Hawkins, J; Omarsdottir, S; Olafsdottir, E.S. *Food Chemistry* **2018**, 245, 989.

Session 5

L13

Chemography and the Biological Activity of Natural Products

Anders Backlund

Uppsala University, UPPSALA, Sweden

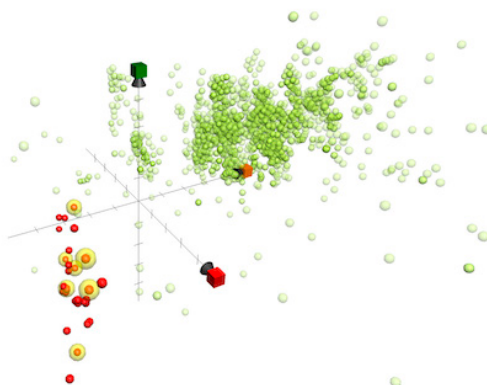
The concept of chemography, navigating chemical space, have over the last decade been applied to a number of studies of natural products .

It has been demonstrated that the concept of proximity in the ChemGPS-NP eight-dimensional chemical property space can be interpreted as a molecular similarity (Rosén et al., 2009), and hence a proxy for the expected biological activity of a particular compound (Buonfiglio et al., 2015).

Combined with methods to define volumes from asymmetric 'clouds' of compound representations, and estimating if a specific compound representation is included in that volume, this provide us with a way of predicting biological activity for a compound – or indicating that an observed activity might be a result of a novel mode of action (e.g. Korinek et al., 2017; Yang et al., 2017, and Xu et al., 2018).

The basis of an efficient exploration of these possibilities, will be the definition of high-quality reference sets for different biological activities, effectively acting as placeholders. In this study examples of applications are discussed.

In the figure is shown a ChemGPS-NP-based analysis for a series of briarane-type diterpenoids active in an inhibition assay of COX-2, and 2,592 previously studied COX-2 inhibitors from the ChEMBL database.



Abstract figure: ChemGPS-NP-based analysis for a series of briarane-type diterpenoids active in an inhibition assay of COX-2.

Reference :

Buonfiglio, R.; Engkvist, O.; Várkonyi, P.; Henz, A.; Vikeved, E.; Backlund, A.; Kogej, T. *Journal of Chemical Information and Modelling* **2015**, *55*, 2375.
 Korinek, M.; Tsai, Y.-H.; El-Shazly, M.; Lai, K.-H.; Backlund, A.; Wu, S.-F.; Lai, W.-C.; Wu, T.-Y.; Chen, S.-L.; Wu, Y.-C.; Cheng, Y.-B.; Hwang, T.-L.; Chen, B.-H.; Chang, F.-R. *Frontiers in Pharmacology* **2017**, *8*, Article 356; doi:10.3389/fphar.2017.00356
 Rosén, J.; Gottfries, J.; Muresan, S.; Backlund, A.; Oprea T. *Medicinal Chemistry* **2009**, *52*, 1953.
 Xu, J.-H.; Lai, K.-H.; Su, Y.-D.; Chang, Y.-C.; Peng, B.-R.; Backlund, A.; Wen, Z.-H.; Sung, P.-J. *Marine Drugs* **2018**, *16*, 75.
 Yang, L.; Chai, C.-Z.; Yan, Y.; Duan, Y.-D.; Henz, A.; Zhang, B.-L.; Backlund, A.; Yu, B.-Y. *Molecules* **2017**, *22*, 1392.

L14

Triterpenoid saponin structure-activity relationships: biological activity, ecological function and evolution

Søren Bak

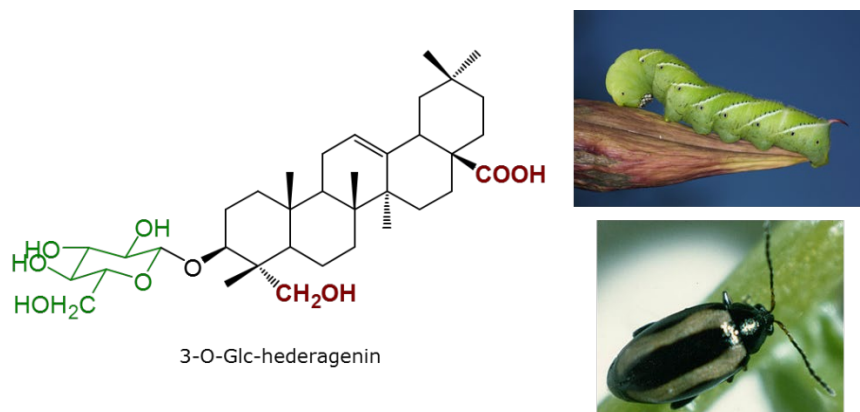
University of Copenhagen, FREDERIKSBERG C, Denmark

Background: Triterpenoid saponins comprise a highly diverse group of specialised metabolites that have evolved recurrently in the plant Kingdom. They are amphipathic molecules thought to be toxic by complexing with membrane sterols.

Methods: We are employing a combination of metabolomics, QTL analysis and NGS sequencing to develop the genus *Barbarea* as a model system for evolution and ecology of chemical defense compounds.

Findings: We have identified a number of genes involved in saponin biosynthesis in *Barbarea vulgaris*. Reconstitution of the pathway in yeast and/or tobacco leaves showed that the pathway enzymes take multiple substrates and produce multiple products, explaining how a few genes can produce the observed structural diversity. A combination of *in planta* pathway reconstitution, *in vitro* enzymatic production, and bioassays, revealed that C22 hydroxylation is important for insect herbivore resistance. CYP72A552 catalyzes this C22 hydroxylation, which causes significant reduced feeding and high larval mortality to the tobacco hornworm and diamondback moth larvae.

Conclusions: Structure-activity relationship studies have unraveled which chemical features of saponins influence toxicity to specific insect herbivores. Our study highlights evolution of chemical novelties by gene duplication, neofunctionalization of enzymes, and the importance of continued chemical modifications as a driver in evolution of plant defense.



Abstract figure for L14

Reference :

Liu Q, Khakimov B, Cárdenas PD, Cozzi F, Olsen CE, Jensen KR, Hauser TP, Bak S. The cytochrome P450 CYP72A552 is key to production of hederagenin-based saponins that mediate plant defense against herbivores. *New Phytol.* 2019.

Byrne SL, Erthmann PØ, Agerbirk N, Bak S, Hauser TP, Nagy I, Paina C, Asp T. The genome sequence of *Barbarea vulgaris* facilitates the study of ecological biochemistry. *Sci Rep.* 2017 7:40728.

Khakimov B, Kuzina V, Erthmann PØ, Fukushima EO, Augustin JM, Olsen CE, Scholtalbers J, Volpin H, Andersen SB, Hauser TP, Muranaka T, Bak S. Identification and genome organization of saponin pathway genes from a wild crucifer, and their use for transient production of saponins in *Nicotiana benthamiana*. *Plant J.* 2015 84(3):478-90.

L15

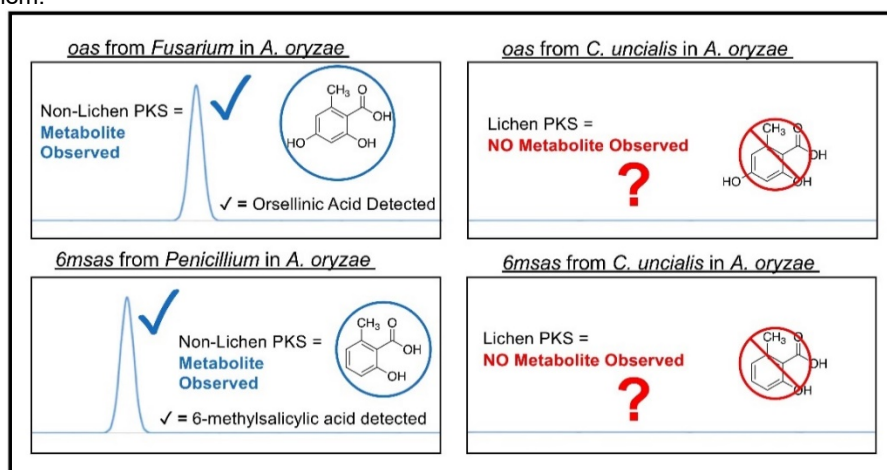
Lichen polyketide synthases - lost in translation: Challenges with heterologous expression in *Aspergillus oryzae*

John Sorensen

University of Manitoba, WINNIPEG, Canada

Lichen fungi remain one of the most underdeveloped sources of novel bioactive natural products.¹ This is due to challenges that result from the slow growth of the symbiotic organism.² Our research program has identified biosynthetic gene clusters in the genome of the lichen *Cladonia uncialis*.³ We have carried out the sequencing, assembly and annotation of the genome and as a result, we have identified approximately 50 biosynthetic gene clusters that code for small molecule biosynthesis.³ A large number of these clusters have a gene that codes for a polyketide synthase. Numerous accessory genes, such as methyl transferases and hydroxylases, flank each of these polyketide synthase genes. Based on homology to genes characterized from non-lichen fungi we have been able to propose function for some of these gene clusters.⁴

We have been using the filamentous fungus *Aspergillus oryzae* as platform for heterologous expression.⁵ We have focused on the gene cluster involved in usnic acid biosynthesis.⁶ This talk will outline the challenges that we have encountered in heterologous expression of biosynthetic genes from *C. uncialis* in *A. oryzae*. We will present some of the challenges that we have encountered in successfully achieving heterologous expression and describe our plans to overcome them.



Abstract: figure for L15

Reference :

- Boustie, J.; Grube, M. *Plant Genetic Resources* **2005**, 3, 273.
Calcott, M. J.; Ackerley, D. F.; Knight, A.; Keyzers, R. A.; Owen, J. G. *Chemical Society Reviews* **2018**, 47, 1730.
Bertrand, R. L.; Abdel-Hameed, M.; Sorensen J. L. *Journal of Natural Products* **2018**, 81, 723.
Bertrand, R. L.; Abdel-Hameed, M.; Sorensen J. L. *Journal of Natural Products* **2018**, 81, 732.
He, Y.; Wang, B.; Chen, W.; Cox, R.; He, J.; Chen, F. *Biotechnology Advances* **2018**, 36, 739.
6. Abdel-Hameed, M.; Bertrand, R.; Piercey-Normore, M.; Sorensen J. L. *Fungal Biology* **2016**, 120, 306

L16

Characterization of the 3-Methyl Orsellinic Acid Biosynthetic Pathway in *Aspergillus nidulans*

Xinhui Wang, Thomas Ostenfeld Larsen, Jakob Blæsberg Hoof, Marie Louise Klejnstrup, Charlotte Held Gotfredsen, Uffe Hasbro Mortensen
Technical University of Denmark, KONGENS LYNGBY, Denmark

It is well known that the filamentous fungus *Aspergillus nidulans* produces 3-methyl orsellinic acid (3-MOA) and cichorine, which are related to the same gene cluster. However, the 3-MOA biosynthetic pathway has only been partially characterized. Especially, some oxidative and reductive steps have not been fully clarified yet. The core polyketide encoding gene *pkbA* is harbored in an eight membered gene cluster, which altogether putatively encode both decorating enzymes and a transcription factor (TF). The fact that *A. nidulans* has three other gene clusters encoding biosynthesis of variants of orsellinic acid altogether complicates the elucidation of the 3-MOA pathway, since cross chemical reactions between the pathways are likely to occur. To get new insights into how 3-MOA is converted to cichorine, we reexamined the mutant strains, with a special focus on knockout of the O-methyltransferase encoding gene. The resulting metabolite profiles were dereplicated based on HPLC-DAD-HRMS analysis. So far several metabolites have been linked to the gene cluster for the first time, and some still uncharacterized likely novel compounds. Altogether, this presentation will provide new genetic and biochemical insights for understanding the structural diversity of this important family of non-reduced polyketides, including a proposed pathway for all identified compounds.

Reference :

- [1] von Döhren H. *Fungal Genetics and Biology* 2009, 46(1): S45-S52.
[2] Nielsen, M. L., Nielsen, J. B., Rank, C., Klejnstrup, M. L., Holm, D. K., Brogaard, K. H., ... & Mortensen, U. H. *FEMS microbiology letters* 2011, 321(2), 157-166.
[3] Ahuja, M., Chiang, Y. M., Chang, S. L., Praseuth, M. B., Entwistle, R., Sanchez, J. F., ... & Wang, C. C. *Journal of the American Chemical Society* 2012, 134(19), 8212-8221.
[4] Sanchez, J. F., Entwistle, R., Corcoran, D., Oakley, B. R., & Wang, C. C. *MedChemComm* 2012, 3(8).
[5] Andersen, M. R., Nielsen, J. B., Klitgaard, A., Petersen, L. M., Zachariasen, M., ... & Mortensen, U. H. *Proceedings of the National Academy of Sciences* 2013, 110(1), E99-E107.

L17

Integrating metabolome and microbiome along a depth gradient in sponges

Karin Steffen¹, Anak Agung Gede Indraningrat², Ida Erngren¹, Jakob Haglöf¹, Paco Cárdenas¹, Detmer Sipkema²

¹Uppsala University, UPPSALA, Sweden

²Wageningen University & Research, WAGENINGEN, Nederland

Sponges are associated with a rich and diverse microbiome and metabolome, and a link between both has been hypothesised. Thus, investigating environmental and microbial contributions to the sponge holobiont natural product production is relevant for bioprospecting and understanding deep-sea ecology.

We assessed microbiome by amplicon sequencing and metabolome by UPLC-HRMS in three deep-sea demosponges (*Geodia barretti*, *Stryphnus fortis*, *Weberella bursa*) sampled along a slope in the North Atlantic Davis Strait (244–1481 m depth) to investigate variations with depth and generate hypotheses about which bacteria produce natural products. We used OPLS models to address microbiome and metabolome variations and built microbial interaction networks to designate candidate producers of the anti-biofouling compound baretin.

We found that depth has an effect on microbiome and metabolome with a shift around 1000 m and signal from putative osmoprotectants increased with depth. Known bioactive compounds for *G. barretti* decreased below 1000 m. From the microbial interaction network, we suggest OTUs belonging to the phyla Chloroflexi, Acidobacteria, Proteobacteria and Gemmatimonadetes as producers for the anti-biofouling compound baretin.

Deep specimens have a unique microbiome and metabolome, and therefore have an inherent potential for future bioprospecting. Integrative omics can help generate hypotheses on natural product production.

Reference :

Manuscript in preparation.

Quantifying variations of microbiota and metabolome composition in deep-sea sponges - implications for chemical ecology and bioprospecting

Karin Steffen, Anak Agung Gede Indraningrat, Ida Erngren, Jakob Haglöf, Leontine E. Becking, Hauke Smidt, Igor Yashayaev, Ellen Kenchington, Paco Cárdenas & Detmer Sipkema.

Session 6

L18

Genomics Driven Discovery and Engineering of Fungal Polycyclic Polyketides

Karolina Subko, Peter B. Wolff, Sebastian Theobald, Jens C. Frisvad, Charlotte H. Gottfredsen, Mikael R. Andersen, Uffe H. Mortensen, Thomas O. Larsen
Technical University of Denmark, KGS. LYNGBY, Denmark

Tetracyclic compounds (TCs) represents an important group of secondary metabolites (SM) exhibiting a broad variety of antimicrobial and cytotoxic activities. In filamentous fungi their backbone structures are synthesised by iterative non-reducing polyketide synthases (NR-PKSs) that undergo regioselective cyclizations as well as further enzymatic modifications. In this study genome sequences of more than 100 species in genus *Aspergillus* were mined against selected polycyclic NR-PKS gene sequences, which identified *A. sydowii* as a promising candidate for novel TC production. However, dereplication by HPLC-DAD-MS analysis showed that the related SM gene cluster was not expressed under standard laboratory conditions. Instead, it was activated by overexpression of a pathway-specific transcription factor leading to the production of multiple SMs. To control the production of SMs related to the pathway we are now using both inducible and constitutive promoters to control the level of the regulatory protein. By construction of deletion strains, supportive real-time quantification PCR, and purification and structural elucidation of significant related products, we are now able to propose a biosynthetic pathway including several branch points and intriguing ring closures.

L19

Bioengineered barley; novel production platform for human proteins

Björn Örvar
ORF Genetics, KÓPAVOGUR, Iceland

ORF Genetics is a biotechnology company with more than 15 years of experience in transgenic technology, hydroponic cultivation and production of endotoxin-free, purified recombinant proteins from plants. The company has developed a novel production platform that can be utilized to produce in barley seeds challenging proteins such as many human growth factors and cytokines. To date, the company has produced over 2.2 million transgenic plants since 2008 and purified (> 94% purity) from the barley seed more than 40 recombinant human proteins, mostly human growth factors for stem cell technology research and skincare products. The company's QMS is based on two ISO standards, or ISO-9001 (QMS Requirements) and ISO-22716 (Cosmetic GMP Guidelines).

Reference :
ORF Genetics

Session 7

L20

Feeding filamentous fungi with halogens - Discovering new natural products from halotolerant, endophytic fungi.

Kenneth Kongstad, Mahdiehalsadat Hosseynimoghadam, Syariful Anam, Louise Kjærulff, Dan Staerk
University of Copenhagen, COPENHAGEN, Denmark

Background Endophytic filamentous fungi inhabiting the intercellular space in plants in a non-pathogenic manner, constitute a promising source of novel bioactive metabolites [1]. Halophilic and halotolerant fungi, capable of growing in high salinity, have previously been shown to produce unique and even halogenated secondary metabolites in response to salt stress [2,3]. Halogenated natural products are rare, but with 30% of all approved small-molecule drugs containing fluorine, inducing the production of halogenated natural products holds great promise.

Methods In this study, endophytes were isolated from halotolerant plants and screened for their ability to grow in saline media. Cultivation-experiments using various salts followed by HPLC-HRMS analyses were used to pinpoint salt-induced secondary metabolites, for subsequent isolation and structural identification by NMR.

Findings HPLC-HRMS analyses of extracts of *Phomopsis azadirachtae* and *Arthrinium arundinis*, both isolated from *Zygophyllum eichwaldii*, cultivated on different halogen-containing media showed the presence of several peaks unique for these halogenated conditions. This led to isolation of several polyketides and a fluorinated secondary metabolite.

Conclusion The study presented here show the potential of investigating halophilic and halotolerant filamentous fungi for discovery of novel secondary metabolites. These findings furthermore indicate an unexplored halogenated chemical space, accessible through systematic cultivation experiments.

Reference :

Strobel, GA. Endophytes as sources of bioactive products. *Microbes Infect.* **2003**, 5, 535-544
Jani, S.; Frisvad, J. C.; Kocev, D.; Gostinar, C.; Džeroski, S.; Gunde-Cimerman, N. Production of secondary metabolites in extreme environments: Food- and airborne *Wallemia* produce toxic metabolites at hypersaline conditions. *PLOS ONE* **2016**, 11, e0169116.
Ali, T.; Inagaki, M.; Chai, H.B.; Wieboldt, T.; Rapplye, C.; Rakotondraibe, L.H.. Halogenated compounds from directed fermentation of *Penicillium concentricum*, an endophytic fungus of the liverwort *Trichocolea tomentella*. *Nat Prod.* **2017**, 80, 1397-1403

L21

Effect of molecular weight on the antimicrobial activity of chitosan and chitosan derivatives.

Már Másson

University of Iceland, REYKJVIK, Iceland

Chitosan is a biocompatible biopolymer that can be obtained by deacetylation of chitin. It has significant antimicrobial properties and this is the basis for many applications. A large number of antimicrobial chitosan derivatives have also been reported.

In the last 10 years, we have reported the synthesis of a series of different chitosan derivatives with a well-defined structure, which have also been analyzed for antimicrobial activity. This has allowed a detailed assessment of the structure-activity relationship and to determine how factors such as substituent structure, distribution, and degree of substitution, the degree of acetylation affect antimicrobial activity¹. The relationship between Mw on the antimicrobial activity of chitosan and chitosan derivatives has been the subject of many studies but there has been no clear consensus on this issue. Here we report a detailed analysis of our own and literature data to show that a general relationship can be defined for the influence of Mw on activity. Our study has shown that the antimicrobial activity of chitosan and chitosan derivatives will increase with Mw until the Critical Molecular Weight for Activity (CMW) is reached and after this point a further increase in Mw will have very limited or no effect on activity.

Reference :

Sahariah, P.; Masson, M., *Biomacromolecules* **2017**, 18 (11), 3846

Session 8

L23

Comparative SAR Study of Common Chitosan Derivatives: Synthesis, Characterization, and Evaluation of Antimicrobial Activity

Sankar Rathinam, Ingibjörg Kristjánsdóttir, Sigríður Jónsdóttir, Priyanka Sahariah, Martha Hjálmarsdóttir, Már Másson
University of Iceland, REYKJAVIK, Iceland

Introduction: The dearth of new antibiotics under clinical investigation raises an urgent call for the development of new potent antimicrobial agents [1, 2]. Chitosan derivatives have been widely studied as promising new anti-bacterial agents. Systematic studies of their structure activity relationship (SAR) and mode of action against bacteria are, however, very rare [4]. The current work focuses on establishing the SAR for some of the widely used chitosan derivatives, and to try to rank them according to activity and utility.

Methods: The synthesis of cationic (TACin, TMC, TMC/DMC and HTCC), anionic (CMC) neutral (HPC) and TGC chitosan derivatives were carried out by using chitosan and TBDMS chitosan as precursors. Structure analysis showed that the side chains were linked to C-2 NH₂ nucleophilic functional group on the polymer backbone.

Conclusion: The chitosan derivatives were characterized by ¹H NMR and FT-IR. All the chitosan derivatives DS were determined to be in the range from (0.04 to 1.4) [5]. We observed a clear relationship between the DS antimicrobial activity, but this depended on structure of the substituent. Cationic derivative had the highest activity against *S. Aureus*, the anionic the lowest and all other derivatives had similar activity against *E. coli*.

Reference :

- [1] Badawy. MEI, Rabea. E, *International Journal of Carbohydrate Chemistry*, **2011**, 1-29
- [2] Priyanka. S, Martha. Á. H, Már. M, *Marine Glycobiology: Principles and Applications*.
- [3] Hancock. R. E, Sahl. H. G, *Nature biotechnology*, **2006**, 24, 1551
- [4] Priyanka. S, Már. M *Biomacromolecules* **2017** 18 3846
- [5] S.P. Rwei, Y. M. Chen, W. Y. Lin W.Y. Chiang, *Mar. Drugs*, **2014**, 12, 5547

The honey bee pollen diet investigated by MS and NMR based metabolomics - floral resource variations and phytochemical fate in hive pollen stores

Nanna Hjørt Vidkjær¹, Jane Ward², Per Kryger¹, Inge S. Fomsgaard¹

¹Aarhus University, SLAGELSE, Danmark

²Rothamsted Research, HARPENDEN, United Kingdom

Declining honey bee (*Apis mellifera*, hereafter bees) populations are concerning because bees are important pollinators of food crops. The decline is hypothesized to be driven by multiple factors, and bees face many stressors including pathogens, xenobiotics and changes in floral resources affecting their diet¹.

Bees consume pollen containing essential nutrients and multitudes of bioactive plant secondary metabolites (PSMs). Recent findings demonstrate the potential of PSMs to affect bee health e.g. by reducing virus loads², but the PSM profile of pollen is sparsely investigated. Different floral resources are available in different environments and throughout the season, but limited knowledge exist on how such variations influences dietary composition.

In a Danish field experiment, biweekly pollen samples (hive entrance and fermented pollen food stores) were collected from four apiaries located in different environments (agricultural, urban, forest and meadow). Chemical profiles of the two types of pollen samples were explored using LC-HRMS and NMR. Seasonal as well as landscape variations in diet composition were subsequently investigated using multivariate data analysis.

The results create a knowledge base for future studies of dietary effects on bee health and allows for novel insight into the chemical fate of PSMs upon hive storage and fermentation prior to consumption.

Reference :

1. Goulson, D., Nicholls, E., Botias, C., Rotheray, E.L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **2015**, 347, 1435.
2. Palmer-Young, E.C., Tozkar, C.O., Schwarz, R.S., Chen, Y., Irwin, R.E., Adler, L.S., Evans, J.D. Nectar and pollen phytochemicals stimulate honey bee (Hymenoptera: Apidae) immunity to viral infection. *Journal of Economic Entomology* **2017**, 110(5):1959.

Session 9

Immunomodulatory polysaccharides from *Inonotus obliquus* (Chaga) and their interaction with pattern recognition receptors

Christian Wold¹, Kari Inngjerdengen¹, Alexandre Corthay², Panagiotis Christopoulos²

¹University of Oslo, OSLO, Norway

²Oslo University Hospital, Rikshospitalet, OSLO, Norway

Background: *Inonotus obliquus* (Chaga), a white rot fungus found on birch trees in the northern hemisphere, has been used in traditional medicine in Europe and Asia for centuries to treat gastro-intestinal disorders, heal wounds and to treat cancer [1]. Recently, in vivo tumor growth-inhibiting polysaccharides were isolated from Chaga [2]. One hypothesis behind the tumor inhibiting effect is that such polysaccharides can activate macrophages in the tumor microenvironment via pattern recognition receptors (PRRs). Depending on which receptors are activated, this could subsequently lead to eradication of tumor cells [3,4]. As such, the present work aims to identify the PRRs responsible for the immunomodulatory effects observed from several polysaccharides isolated from Chaga [5].

Results: Several polysaccharides from Chaga were able to induce in vitro tumor growth inhibition indirectly by activating macrophages, and induce production of nitric oxide and pro-inflammatory cytokines IL-6 and TNF- α in these cells. The receptors responsible for the macrophage activation were searched for using bone-marrow derived macrophages from mice deficient in different PRRs (e.g. TLR4^{-/-}, MyD88^{-/-} and Dectin-1^{-/-}), and an array of antagonist experiments blocking various receptors. Preliminary results show that the polysaccharides bind to more than one type of PRR, including TLR-4 and possibly Dectin-1.

Reference :

- [1] Zheng W. et al., Chemical diversity of biologically active metabolites in the sclerotia of *Inonotus obliquus* and submerged culture strategies for up-regulating their production, *Appl Microbiol Biotechnol* 2010, 87: 1237-1254.
- [2] Chen Y. et al., Purification, characterization and biological activity of a novel polysaccharide from *Inonotus obliquus*, *Int J Biol Macromol* 2015, 79: 587-594.
- [3] Müller E. et al , Toll-Like Receptor Ligands and Interferon- γ Synergize for Induction of Antitumor M1 Macrophages., *Front. Immunol.* 2017, 8:1383.
- [4] Erwig L.P & Gow, N.A, Interaction of fungal pathogens with phagocytes, *Nat Rev Microbiol* 2016, 14(3): 163-176.
- [5] Wold C. et al., Structural characterization of bioactive heteropolysaccharides from the medicinal fungus *Inonotus obliquus* (Chaga)., 2018, 185: 27-40.

L26

Effect of Falcarindiol and Falcarinol Purified from Carrots on Inflammation and Cancer

Eva Arnspang¹, Emma Pipó-Ollé¹, Martine Notabi¹, Martin Hedegaard¹, Anders Walther¹, Morten Kobaek-Larsen¹, Gunnar Baatrup¹, Rime El-Houri¹, Lars Porskjaer Christensen²

¹University of Southern Denmark, ODENSE, Denmark

²Aalborg University, AALBORG, Denmark

Falcarinol (FaOH) and falcarindiol (FaDOH) are cytotoxic and anti-inflammatory polyacetylenic oxylipins found in food plants of the carrot family (Apiaceae). The cytotoxic effect of FaOH and FaDOH is synergistically enhanced in combination and recently an anticancer effect has been shown in rats. In this study, we have done a study of selected biomarkers in rat tissue and in mammalian cell culture relevant for colon cancer and found downregulation of COX-2 in rat tissue receiving FaOH and FaDOH as well as in cell culture. Furthermore, we have studied the impact of FaDOH in cancer cells and found formation of a cholesteryl ester and lipid droplets. This was verified with fluorescence microscopy and Raman imaging.

Reference :

Morten Kobaek-Larsen, Gunnar Baatrup, Martine K. Notab, Rime B. El-Houri, Emma Pipó-Ollé, Eva C. Arnspang and Lars P. Christensen, Impact of the Dietary Polyacetylenic Oxylipins Falcarinol and Falcarindiol on Inflammation and Colorectal Cancer: A Dose-Response and Mechanistic Study in a Primed Rat Model, In review in Cancer Prevention Research.

L27

Did we have enough evidence to describe galantamine as an allosteric modulator of nicotinic receptors?

Natalia Magdalena Kowal¹, Philip K Ahring², Vivian W Y Liao², Dinesh C Indurthi², Benjamin S Harvey³, Susan M O'Connor³, Mary Chebib², Elín S Ólafsdóttir¹, Thomas Balle²

¹University of Iceland, REYKJAVIK, Iceland

²The University of Sydney, SYDNEY, Australia

³Bionomics Limited, THEBARTON, Australia

Introduction: Galanthamine, a plant alkaloid isolated from snowdrop (*Galanthus* sp.), is approved as a drug for treatment of mild-to-moderate Alzheimer's disease. Galanthamine works primarily as an acetylcholinesterase inhibitor but it is also commonly referred to as a positive allosteric modulator (PAM) of neuronal nicotinic acetylcholine receptor (nAChR). Previous experiments that showed galantamine as a PAM at nAChR were primarily conducted on rat hippocampal neurons¹, PC12² cells naturally expressing nAChRs and in HEK cells³ transfected with nAChR subunits. Data available from receptors expressed in *Xenopus* oocytes are limited and show marginal PAM activity. The aim of the study was to answer the question: Is galantamine really a PAM of nAChRs?

Methods: Various subtypes and stoichiometries of human neuronal nAChRs were expressed in *Xenopus* oocytes. Electrophysiological currents evoked by ACh alone or in combination with galantamine were recorded using two electrode voltage clamp.

Results: Galanthamine was unable to produce significant PAM responses when tested on $\alpha 7$, $\alpha 4\beta 2$ and $\alpha 4\beta 4$ nAChRs expressed in *Xenopus* oocytes. However, in agreement with the literature we observed inhibition of ACh-evoked responses at high concentrations (10 – 100 μ M range) which is a result of an open pore blockade.

Conclusions: Galanthamine is not a PAM of nAChRs.

Reference :

Pereira, E. F.; Alkondon, M.; Tano, T.; Castro, N.; Froes-Ferrao, M.; Rozental, R.; Aronstam, R.; Schrattenholz, A.; Maelicke, A.; Albuquerque, E., A novel agonist binding site on nicotinic acetylcholine receptors. *Journal of receptor research* **1993**, *13* (1-4), 413-36. Storch, A.; Schrattenholz, A.; Cooper, J.; Abdel Ghani, E.; Gutbrod, O.; Weber, K.; Reinhardt, S.; Lobron, C.; Hermesen, B.; Soskic, V.; et al., Physostigmine, galanthamine and codeine act as 'noncompetitive nicotinic receptor agonists' on clonal rat pheochromocytoma cells. *European journal of pharmacology* **1995**, *290* (3), 207-19. Samochocki, M.; Zerlin, M.; Jostock, R.; Groot Kormelink, P.; Luyten, W.; Albuquerque, E.; Maelicke, A., Galantamine is an allosterically potentiating ligand of the human $\alpha 4\beta 2$ nAChR. *Acta Neurologica Scandinavica* **2000**, *102*, 68-73.

Poster session 1

P01

Analysis of the content of fucoxanthin in *Fucus vesiculosus* and *Laminaria digitata* from Iceland.

Signe Ptak, Bjarke Andersen, Toke Murmann, Xavier Fretté
University of Southern Denmark, ODENSE, Denmark

Background: Fucoxanthin is the most abundant carotenoid in brown algae¹ and has been shown to exert certain biological activities that are beneficial to humans². Quantifying the fucoxanthin amount in algae is essential for establishing to what extent the algae have any health effects upon consumption. The extraction of fucoxanthin is highly affected by the extraction solvent, however. This work presents the quantification of fucoxanthin in *Fucus vesiculosus* (FV) and *Laminaria digitata* (LD) collected in Iceland using dichloromethane, acetone, ethanol and methanol.

Methods: The dried algae (1g) were extracted with 10 mL solvent over 1 h. The extraction was repeated 2 times. The extracts were filtered into HPLC vials for HPLC analysis (C18 column, gradient elution with water:acetonitrile). Fucoxanthin was quantified using a linear calibration curve in the range 116 µg/mL – 0.4 µg/mL ($R^2=0.9998$). Analyses were performed in triplicate.

Results: The amount of fucoxanthin was determined to be 400, 232, 245 and 228 µg/g DW FV, and 273, 153, 158 and 125 µg/g DW LD when extracted with dichloromethane, acetone, ethanol and methanol, respectively.

Conclusion: Reference articles on fucoxanthin extraction suggest ethanol as the best solvent for fucoxanthin extraction^{3,4}. Our results show that dichloromethane achieves better yields.

Reference :

Kumar, S.R.; Hosokawa, M.; Miyashita, K. Marine Drugs **2013**, *11*, 5130.
Jung, H.A.; Ali, M.Y.; Choi, R.J.; Jeong, H.O.; Chung, H.Y.; Choi, J.S. Food Chem. Toxicol. **2016**, *89*, 104.
Kim, S.M.; Kang, S.W.; Kwon, O.N. J. Korean Soc. Appl. Biol. Chem. **2012**, *55*(4), 477.
Shannon E., Abu-Ghannam N., J. Appl. Phycol. **2016**, *28*, 1.

P02

'Paper-based biofilm substrates as novel tools for anti-biofilm drug discovery from environmental sources'

Paola San Martin Galindo¹, Jussi Kauppila², Emil Rosqvist², Jouko Peltonen², Adyary Fallarero¹

¹University of Helsinki, HELSINKI, Finland

²Abo Akademi University, TURKU, Finland

Bacterial biofilms are characterized by an increased resistance to antimicrobial therapy, when compared to the single-cells¹. The need for new anti-biofilm compounds is at all-times high. The recent discovery of a potent antibiotic, teixobactin, from soil uncultured bacteria, has renewed the interest for the exploration of environmental sources². In particular, high load and variety of microorganisms are typically presented in waste sources, which contain abundance of antibiotic resistance genes and likely a large number of unidentified bioactive metabolites³. In this project, the first steps were taken towards the exploration of new anti-biofilm compounds from waste, by developing a low-cost biofilm sensor. The biosensor can identify antibacterial-active sites from waste water, and potentially lead to easier prospecting of unique antimicrobial metabolites. The developed biofilm sensor was based on paper-based arrays substrates which have been physico-chemically characterized in relation to both biofilm attachment and stability. Based on these studies, the best substrate was selected and it is planned to be placed in selected waste sites. Subsequently, the biosensor's surface will be subjected to specific microbiological assays to identify anti-biofilm and/or matrix-degrading bacterial strains. Discovering bioactive metabolites in their original growth environment, increases the probability for untapping new valuable chemotypes as drug leads.

Reference :

1. Olsen, I. Eur J Clin Microbiol Infect Dis 2015, *34*, 877.
2. Losee L, Schneider T, Peoples A, Spoering A, Engels I, et al. Nature 2015, *517*, 455.
3. Chen Q, An X, Li H, Su J, Ma Y, Zhu YG. Environ Int 2016, *92-93*, 1.

P03

Characterization of the activity of three natural products as potential antimicrobials against *Staphylococcus aureus*

Shella Gilbert-Girard, Kirsi Savijoki, Jari Yli-Kauhaluoma, Adyary Fallarero
University of Helsinki, HELSINKI, Finland

Background: Biofilms are static colonies of bacteria attached to a surface and embedded in a polysaccharide matrix. They display a much higher tolerance to antibiotics and can lead in humans to chronic infections unsuceptible to treatment. Bacteria are also developing resistance against existing antimicrobials, underlining the need for new anti-biofilm molecules. Natural products have been used as medication for many purposes and they are still a popular

source of compounds in anti-infective research.

Methods and findings: We have screened a natural products library containing 500 investigational and approved compounds in search of new repurposed antimicrobials effective against the human pathogen *Staphylococcus aureus*. Three molecules have shown potent activity in killing staphylococcal cells and completely preventing biofilm formation. The compounds also had a great bactericidal effect on pre-formed biofilms and showed activity against different gram-positive and gram-negative bacterial strains. A chemoinformatics analysis was made using the ChemGPS-NP tool to compare the chemical space occupied by the hits and understand the connections between their biological activity and their physico-chemical properties.

Conclusion: These three natural products were selected as promising anti-biofilm leads and will be considered for integration into polymer formulations that could be used to produce prototypes of anti-infective 3D-printed medical devices.

Reference :

- Olson, M.E., et al. *Can J Vet Res* **2002**. 66(2), p. 86.
Anwar, H., M.K. Dasgupta, and J.W. Costerton. *Antimicrob Agents Chemother* **1990**. 34(11), p. 2043.
Davies, D. *Nat Rev Drug Discov* **2003**. 2(2), p. 114.
Costerton, J.W., P.S. Stewart, and E.P. Greenberg. *Science* **1999**. 284(5418), p. 1318.
Boucher, H.W., et al. *Clin Infect Dis* **2009**. 48(1), p. 1.
Spellberg, B., et al. *Clin Infect Dis* **2008**. 46(2), p. 155.
Skogman, M.E., P.M. Vuorela, and A. Fallarero. *J Antibiot (Tokyo)* **2012**. 65(9), p. 453.
Larsson, J., J. Gottfries, S. Muresan and A. Backlund. *J Nat Prod* **2007**. 70(5), p. 789.
Rosen, J., J. Gottfries, S. Muresan, A. Backlund and T. I. Oprea. *J Med Chem* **2009**. 52(7), 1953.

P04

Biofilm-inhibiting effects of two Dehydroabietic acid derivatives on clinically relevant surfaces in the presence of host cells

Inés Reigada, Kirsi Savijoki, Jari Yli-Kauhaluoma, Adyary Fallarero
University of Helsinki, HELSINKI, Finland

Implantation of a foreign body is the starting point of a competition between cells of tissues and biofilm forming bacteria¹. Previously, combining the abietane moiety of dehydroabietic acid (DHA) with different amino acids was shown to result in a new class of hybrid compounds able to efficiently target *Staphylococcus aureus* biofilms². Here, the two most effective of these DHA derivatives were tested *in vitro* under conditions simulating those faced *in vivo* to investigate their ability to prevent *S. aureus* biofilm formation on titanium coupons in the presence of mammalian cells (SaOS-2). For that purpose, a co-culture of both *S. aureus* (ATCC 25923) and SaOS-2 was added to a titanium coupon coated with these DHA derivatives (coded 11 and 9b). After 24 hour of incubation, neither one of the compounds caused cytotoxicity, but pre-exposure to compound 11 caused a significant inhibition of biofilm formation. These selective inhibitory effects on *S. aureus* were also maintained when the coupons were exposed to 11 and both cellular systems (*S. aureus* and SaOS-2) at the same time. In conclusion, our results indicate that compound 11 shows promise as a selective biofilm inhibiting agent and could protect medical devices from *S. aureus* infection.

Reference :

- Perez-Tanoira R, Han X, Soininen A, Aarnisalo AA, Tiainen VM, Eklund KK, et al. Competitive colonization of prosthetic surfaces by staphylococcus aureus and human cells. *J Biomed Mater Res Part A*. 2017;105:62. Manner S, Vahermo M, Skogman ME, Krogerus S, Vuorela PM, Yli-Kauhaluoma J, et al. New derivatives of dehydroabietic acid target planktonic and biofilm bacteria in *Staphylococcus aureus* and effectively disrupt bacterial membrane integrity. *European journal of medicinal chemistry*. 2015;102:68.

P05

Evaluation of phytochemical profile and potential biological activities of methanol extracts from Bryophytes

Raissa Volpatto Marques¹, Sissi Miguel², Frédéric Bourgaud², Henrik Toft Simonsen¹

¹Technical University of Denmark, KONGENS LYNGBY, Denmark

²Plant Advanced Technologies, VANDŒUVRE-LÈS-NANCY, France

Bryophytes contain remarkable source of small molecules with high biological activity. However, few species had their biological properties well investigated. Thus, they are an important source of promising compounds for medicine, cosmetic field and agriculture. The aim of this study is to analyze the composition of small molecules from a variety of bryophytes species and to identify molecules with relevant bioactivity. In a preliminary study, methanol extracts from 12 species (11 mosses and 1 liverwort) had their phytochemical profile investigated by UPLC-MS and the total concentration of polyphenols evaluated by the Folin-Ciocalteu method. In addition, their antioxidant activity was evaluated by DPPH radical scavenging method and anti-tyrosinase activity screened using mushroom tyrosinase inhibition method. The following groups of most abundant compounds were identified: flavonoids, terpenoids and phenylpropanoids. In comparison, the liverwort from the family Lepidoziaceae presented the highest content of polyphenols 0.8 mg/ml of gallic acid equivalent (GAE) and the strongest antioxidant activity (DPPH IC₅₀, 0.10 g/L). Similarly, two moss extracts from the families Polytrichaceae and Hylocomiaceae exhibited a considerable level of

59% tyrosinase inhibition. This study presents potential new biological activities in not well-studied bryophytes. Other species extracts will be screened and bioactive compounds identified via Targeted binding® technology.

P06

Magnetic ligand fishing coupled with HPLC-PDA-HRMS-SPE-NMR: proof of concept and identification of antidiabetic bi-flavonoids from *Ginkgo biloba*

Malene J Petersen, Dan Staerk
University of Copenhagen, COPENHAGEN, Denmark

Background Type 2 diabetes is a metabolic disorder affecting close to 400 million people worldwide [1], and natural products constitute a promising source for new anti-diabetic drug leads. High-resolution bioactivity profiling coupled with HPLC-PDA-HRMS-SPE-NMR has been successfully used for identification of inhibitors of T2D-related enzymes [2,3], but complementary techniques with improved sensitivity and resolution is desired.

Methods Magnetic beads with immobilized α -amylase (on 3-carbon linker) or α -glucosidase (on 3- or 21-carbon linker) were used for ligand-fishing, and HPLC-PDA-HRMS-SPE-NMR was used for subsequent structural characterisation of the ligands.

Findings α -Amylase and α -glucosidase were successfully immobilized onto magnetic beads [4]. Subsequent stability experiments showed that the enzymes retained catalytic activity upon 76 days of storage (α -amylase) and after eight successive reuses of the beads (α -glucosidase). Proof-of-concept studies with extracts of *Ginkgo biloba* fished out four α -amylase ligands and seven α -glucosidase ligands from the extract. Bi-flavonoids fished out with α -glucosidase-immobilized beads possessed α -glucosidase inhibitory activity with IC_{50} -values from 16.4 to 68.2 μ M.

Conclusion Ligand fishing with α -amylase- and α -glucosidase-immobilized beads resulted in identification of several ligands of the two enzymes, and ligand fishing is an effective method for identification of new α -amylase and α -glucosidase ligands from crude extracts.

Reference :

International Diabetes Federation. IDF Diabetes Atlas, 8th edition, 2017. ISBN: 978-2-930229-87-4.
<http://www.diabetesatlas.org/> Accessed 2019-02-26.

Okutan, L., Kongstad, K.T., Jäger, A.K., Staerk, D. High-resolution α -amylase assay combined with high-performance-liquid-chromatography-solid-phase extraction-nuclear magnetic resonance-spectroscopy for expedited identification of α -amylase-inhibitors: proof-of-concept and α -amylase inhibitor in cinnamon. *Agric. Food Chem.* 2014, 62, 11465-11471.

Wubshet, S.G., Moresco, H.H., Tahtah, Y., Brighente, I.M.C., Staerk, D. High-resolution bioactivity-profiling combined with HPLC-HRMS-SPE-NMR: α -glucosidase inhibitors and acetylated ellagic acid rhamnosides from *Myrcia palustris* (Myrtaceae). *Phytochemistry* 2015, 116, 246-252.

Wubshet, S.G.; Liu, B.; Kongstad, K.T.; Böcker, U.; Petersen, M.J.; Li, T.; Wang, J.; Staerk, D. Combined magnetic ligand fishing and high-resolution inhibition-profiling for identification of α -glucosidase inhibitory ligands: A new screening-approach based on complimentary inhibition and affinity-profiles. *Talanta* 2019, in press.

P07

Surveying eight *Eremophila* species for novel antihyperglycemic and antibacterial compounds using bioactivity profiling combined with HPLC-PDA-HRMS-SPE-NMR.

Hans Albert Pedersen¹, Susan Semple², Nhung Thi Dinh¹, Dan Staerk¹

¹University of Copenhagen, COPENHAGEN, Denmark

²University of South Australia, ADELAIDE, Australia

Background Type 2 diabetes and multidrug-resistant bacteria are major and increasingly serious health concerns worldwide [1,2]. Plant natural products are a promising source of the new drug leads that are urgently needed to combat both.

Methods Resins and leaf extracts from eight *Eremophila* species were screened for potential antihyperglycemic and antibacterial compounds using high-resolution bioactivity profiling and subsequently analyzed by HPLC-PDA-HRMS-SPE-NMR [3] to prioritize structural elucidation of active compounds.

Findings Screening of resins and leaf extracts in three antidiabetic enzyme assays revealed most resins were active ($> 50\%$ inhibition at $100 \mu\text{g mL}^{-1}$) against PTP1B, while the leaf extract of *E. altemifolia* alone inhibited α -glucosidase, α -amylase, and PTP1B. Most resins and some leaf extracts showed activity ($MIC < 128 \mu\text{g mL}^{-1}$) against *S. aureus*. The first compounds identified in this study include serrulatanes, iridoids, and phenylpropanoids. Standardized HPLC methods and fractionation procedures were concurrently developed to facilitate HRMS-based dereplication across *Eremophila* species and extracts.

Conclusion *Eremophila* species vary widely in antidiabetic and antibacterial potential, but resins appear to be the more promising source for bioactive compounds in both cases. Efficient HPLC-HRMS/MS dereplication strategies will help provide a thorough description of the phytochemical complexity.

References

International Diabetes Federation. IDF Diabetes Atlas, 8th edition, **2017**. ISBN: 978-2-930229-87-4.
<http://www.diabetesatlas.org/> Accessed 2019-02-26.
O'Neill, J. 2016 Tackling drug-resistant infections globally: Final report and recommendations. The Review on Antimicrobial Resistance. <https://amr-review.org> Accessed 2019-02-26
Tahtah, Y.; Wubshet, S.G.; Kongstad, K.T.; Heskes, A.M.; Paternaki, I., Møller, B.M., Staerk, D. High-resolution PTP1B inhibition profiling combined with high-performance liquid chromatography-high-resolution mass spectrometry-solid-phase extraction-nuclear magnetic resonance spectroscopy: Proof-of-concept and antidiabetic constituents in crude extract of *Eremophila lucida*. *Fitoterapia* **2016**, *110*, 52.

P08

Greek mountain tea, a medicinal plant from Mediterranean countries and Balkan

Nastaran Moussavi

School of Pharmacy, University of Oslo, OSLO, Norge

Background: Greek mountain tea, *Sideritis scardica*, has a long history in traditional medicine. Currently, *S. scardica* is investigated for its pharmacological activity in the central nervous system in which cognition-enhancing and neuroprotective properties have been described (1-2).

Methods: Dried leaves of *S. scardica* were extracted with 80 % ethanol, then the extract was suspended in distilled water and successively extracted with solvents of increasing polarity. Selected extracts were fractionated by different chromatographic techniques and isolated compounds identified by NMR spectroscopy. Isolated compounds and extracts were tested in antioxidant and anti-inflammatory *in vitro* systems, as well as in cholinesterase inhibition assays.

Finding: The 80% ethanol extract was found to contain flavones (glycosides of isoscutellarein and hypolaetin), phenylethanoids (mainly verbascoside) and chlorogenic acid as the main constituents. Antioxidant and anti-inflammatory effects were ascribed to the high content of polyphenols in the 80% ethanol extract. No inhibition of acetylcholine- or butyrylcholinesterases was observed.

Conclusion: Antioxidant and anti-inflammatory effects are suggested to play a protective role in the pathogenesis of neurodegenerative diseases. Our findings may seem to be in compliance with previous *in vivo* findings and should be followed up in future studies.

Reference :

1: Hofrichter J et al., *J Alzheimer's Dis* **2016**, *53*, 967-980, 2: Heiner F et al., *PeerJ* **2018**, *6*, e4683

P09

Searching for the transcriptional regulators of saponin biosynthesis in *Barbarea vulgaris*

Pablo D. Cardenas, Karen Rysbjerg, Thure Hauser, Søren Bak

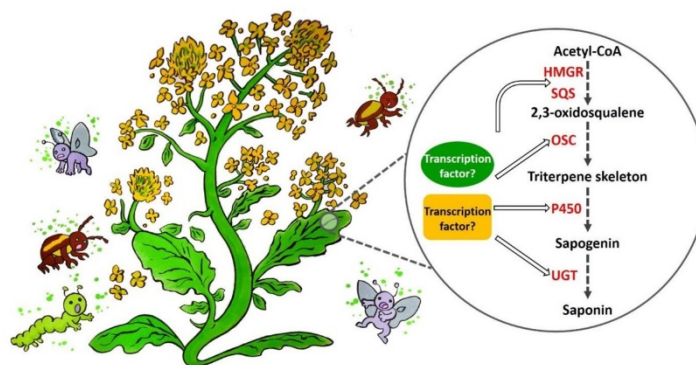
University of Copenhagen, FREDERIKSBERG, Danmark

Background: There is a critical need to develop novel and sustainable methods to reduce losses in agriculture caused by insect pests and pathogens. Plants produce a blend of specialized chemicals that deter antagonists, whose potential for pest management is still largely unexplored. We need to better understand how such defense compounds are synthesized by plants, in order to develop and use them sustainably.

Methods: We are exploring the molecular regulation of triterpenoid saponin biosynthesis, using the insect-resistant wild crucifer *Barbarea vulgaris* as a model system. We use a combination of metabolomics, QTL analysis and NGS sequencing to uncover this process.

Findings: Using co-expression analysis and fine mapping of previously reported QTLs associated with resistance to herbivory, we have identified putative transcriptional factors regulating saponin biosynthesis. Currently, we are generating plant lines with altered expression of these regulators in order to explore their role. Additionally, we are investigating how saponin biosynthesis and its regulation is affected by herbivore attacks using the larvae of the diamondback moth, *Plutella xylostella*.

Conclusion: Our findings will provide insight into the regulation of saponin biosynthesis and means for manipulating these metabolites to develop crops with modified content of saponins and ecologically appropriate defense reactions.



Abstract figure for P09

Reference :

Khakimov B.; Kuzina V.; Erthmann P.Ø.; Fukushima E.O.; Augustin J.M.; Olsen C.E.; Scholtalbers J.; Volpin H.; Andersen S.B.; Hauser T.P.; Muranaka T.; Bak S. *Plant Journal* **2015**, 84, 478.
 Byrne, S.L.; Erthmann P.Ø.; Agerbirk N.; Bak S.; Hauser T.P.; Nagy I.; Paina C.; Asp T. *Scientific Reports* **2017**, 7, 40728.
 Liu Q.; Khakimov B.; Cárdenas P.D.; Cozzi F.; Olsen C.E.; Jensen K.R.; Hauser T.P.; Bak S. *New Phytologist* **2019**, doi: 10.1111/nph.15689.

P10

IN VITRO MICROPROPAGATION OF MOSSES IN A TEMPORARY IMMERSION SYSTEM (TIS) PLATFORM BIOREACTOR

Isidora Loncarevic¹, Finnur Freyr Eiriksson², Margrét Þorsteinsdóttir³, Nils Cronberg⁴

¹Lund University/ArcticMass, REYKJAVIK, Sweden

²ArcticMass, REYKJAVIK, Iceland

³University of Iceland, REYKJAVIK, Iceland

⁴Lund University, LUND, Sweden

In vitro propagation of plants is important for obtaining a large quantity of biomass. Temporary immersion systems (TIS) has been recognized as a propitious technology for plant micropropagation, but also for production of specialized metabolites. However, experience of using the Plantform bioreactor, as TIS, for axenic cultivation of mosses is lacking. The objective is to assess this bioreactor for micropropagation of selected bryophyte species in comparison to conventional culturing on agar plates, by conducting growth analysis. The Plantform bioreactor has been designed to be easy to handle and to reduce time-consuming manual work. By forced ventilation in Plantform, gas exchange is improved, which minimizes the difference between the gaseous environment in vitro and ex vitro. Also, plants have controlled access to nutrients, in theory more efficiently compared to solid media. Frequency and duration of immersion and aeration are considered as the most critical parameters for system efficiency. Our initial tests show that this type of TIS bioreactor performs better than agar plates for moss culture growth when plants are exposed to the same culturing media and light conditions.

P11

Bioactive triterpenoids from *Inonotus obliquus* (Chaga)

Christian Wold¹, Kari Inngjerdengen¹, William Gerwick², Helle Wangenstein¹

¹University of Oslo, OSLO, Norway

²Scripps Institution of Oceanography, LA JOLLA, SAN DIEGO, United States of America

Background: *Inonotus obliquus* (Chaga), a fungus growing on birch trees in the Northern hemisphere, has been used traditionally in Europe and Asia for centuries to treat gastro-intestinal disorders, heal wounds and to treat cancer [1]. Bioactive triterpenoids such as betulinic acid and inotodiol have been isolated from Chaga, and most of the research has focused on the cytotoxic effects of the triterpenoids in cancer cells [2]. As such, our current work has explored new types of bioactivities of triterpenoids isolated from Chaga.

Methods: Six terpenoids were isolated from Chaga using column chromatography, TLC and HPLC. The terpenoids were screened for a wide range of in vitro activities, including anti-complement activity, inhibition of the enzymes xanthine oxidase, 15-LO and acetylcholinesterase, anti-inflammatory activity in macrophages, viability of cancer cells (MTT) and radical scavenging of DPPH.

Results: One triterpenoid not previously found in Chaga, betulin-3-O-cafeate, as well as five known triterpenoids were isolated. Betulin-3-O-cafeate displayed potent anti-inflammatory and radical scavenging properties, and inhibited 15-LO. 3β-Hydroxy-lanosta-8,24-dien-21-al strongly interacted with human complement proteins (IC₅₀=1.1 μM). Betulinic acid reduced viability of the methotrexate-resistant cancer cell line HT29-MTX (IC₅₀=0.6 μM).

Conclusions: Several triterpenoids isolated from Chaga have bioactive properties in vitro, including

immunomodulatory and antioxidant activity.

Reference :

[1] Zheng W. et al., Chemical diversity of biologically active metabolites in the sclerotia of *Inonotus obliquus* and submerged culture strategies for up-regulating their production, *Appl Microbiol Biotechnol* 2010, 87: 1237-1254. [2] Taji S. et al., Lanostane-type triterpenoids from the sclerotia of *Inonotus obliquus* possessing anti-tumor promoting activity., *Eur J Med Chem* 2008, 43(11):2373-2379.

P12

Ethnopharmacology, biological activities and chemical compounds of *Canarium strictum*: an important resin-yielding medicinal tree in India

Gopalakrishnan S. Seethapathy¹, Kaliamoorthy Ravikumar², Hugo J. De Boer³, Helle Wangensteen¹

¹Section for Pharmaceutical Chemistry, Department of Pharmacy, University of Oslo, OSLO, Norway

²The Institute of Trans-Disciplinary Health Sciences and Technology, BANGALORE, India

³Natural History Museum, University of Oslo, OSLO, Norway

Background: *Canarium strictum* is a tree distributed across parts of India, Myanmar and China. The resin is used for rheumatism, asthma, venereal disease and chronic cutaneous diseases; the bark is used as mosquito repellent. Triterpenoids are reported to be the major compounds in the resin, no phytochemical studies have been performed on the bark.

Methods: Ten folk healers aged 45 to 67 in Western Ghats, India were interviewed about their medicinal use of *C. strictum*. The resin and stem bark extracts were fractionated using different chromatographic methods, and isolated compounds were identified by NMR.

Findings: All interviewed healers employed resin, and the reasons for use were related to cold, airways diseases and rheumatoid arthritis. α - and β -amyrin, and lupeol were identified in the resin extract. From the stem bark, procyanidins, flavan-3-ols, gallic acid derivatives, a coumarin and an ellagic acid glycoside were isolated. Antioxidant activity were observed in resin and bark extracts, no toxicity towards *A. salina nauplii* was found.

Conclusion: Triterpenoids and procyanidins are the major compounds in *C. strictum* resin and stem bark, respectively. The high content of triterpenoids might contribute to anti-inflammatory effects and give a rationale for the wide spread usage of the resin in India.

P13

Hidden in the barks: chemical analyses of *Cinchona* herbarium specimens

Tobias Nikolaj Gress Hansen¹, Nataly Allasi Canales¹, Christopher J. Barnes¹, Kim Walker², Mark Nesbitt², Alexandre Antonelli², Claus Cornett³, Nina Rønsted¹

¹Natural History Museum of Denmark, COPENHAGEN, Denmark

²Royal Botanic Gardens, KEW, RICHMOND, United Kingdom

³Dept. of Pharmacy, COPENHAGEN, Denmark

The barks of trees belonging to the genus *Cinchona* have been used as treatment for malaria since its discovery in Peru in the 17th century.

The antimalarial compounds are alkaloids found in the bark with quinine being the most widely used, but more than 30 related compounds are described from the genus. Although quinine has largely been replaced by synthetic antimalarials, renewed interest and research into quinine and its related compounds has arisen, due to parasite resistance to newer drugs.

This presentation is based on quantitative High Pressure-Liquid Chromatography (HPLC) analysis of the four major alkaloids from 100+ bark samples from the historical collections in Kew Gardens, UK. The samples were collected from the early 1800s from a variety of both natural localities and plantations. Remarkably, the alkaloids are still intact and provide a unique record of the early history of *Cinchona* barks.

The chemical results will be correlated with phylogeny and locality and aid in the understanding of which factors control the chemical production in the barks. The HPLC profiles also contain information regarding yet unidentified, possibly pharmacologically relevant, compounds.

Reference :

Andersson, L. (1998). A Revision of the Genus *Cinchona* (Rubiaceae-Cinchoneae), Memoirs of the New York Botanical Garden, vol. 80/81.

Holmfred, E., Cornett, C., Maldonado, C., Rønsted, N., & Hansen, S. (2017). An Optimised Method for Routine Separation and Quantification of Major Alkaloids in Cortex *Cinchona* by HPLC Coupled with UV and Fluorescence Detection. *Phytochemical Analysis*, 28(5), 374–380. <https://doi.org/10.1002/pca.2684>

Maldonado, C., Barnes, C. J., Cornett, C., Holmfred, E., Hansen, S. H., Persson, C., ... Rønsted, N. (2017). Phylogeny Predicts the Quantity of Antimalarial Alkaloids within the Iconic Yellow *Cinchona* Bark (Rubiaceae: *Cinchona calisaya*). *Frontiers in Plant Science*, 8, 536. <https://doi.org/10.3389/fpls.2017.00391>

P14

Gelatin films for drug delivery

Svetlana Solodova, Kristinn Páll Sigurbjörnsson, Már Masson
University of Iceland, REYKJAVIK, Island

Introduction: Gelatin (GT) is a natural protein derived from the hydrolysis of collagen. It is a biocompatible, biodegradable and multifunctional biopolymer which is widely used in food, pharmaceutical, cosmetic and medical applications due to its unique mechanical and technological properties¹⁻². The purpose of the study was to investigate the properties of mammalian and fish gelatin and its potential application as drug delivery systems.

Methods: The films production was optimized using sorbitol as plasticizer and ferulic acid as a cross-linker. GT-films solubility, swelling degree were evaluated. Mechanical properties of GT-films were investigated by a texture analyzer. Drug release profiles with model drug Na-diclofenac were obtained by Franz-cell experiments and were measured by HPLC-analysis.

Results and conclusion: Different types of GT-films were investigated. It was obtained that using plasticizer and cross-linker has effect on physical and mechanical properties of GT-films. It was observed that drug release from fish skin GT-films is faster compare to mammalian skin GT-films, what is a desirable property for many applications. The modification of GT and its combinations with plasticizers and cross-linkers have demonstrated the maneuverability of finding required delivery systems that enable specific, targeted and controlled release in response to demands in the body.

Reference :

Rao K.P. Recent developments of collagen-based materials for medical applications and drug delivery systems. J Biomater Sci Polym Ed, 1995; 7, 623-45
Foux M., Zilberman M. Drug delivery from gelatin-based Systems. Expert Opin. Drug Deliv., 2015, 12(9), 1547-1563

Poster session 2

P15

Species identification and adulteration of Concentrated Chinese Medicine Granules applied in women's healthcare on the European market

Felicitas Mück¹, Helle Wangenstein¹, Hugo De Boer¹, Kristin Odlo Høye², Martin Fitzgerald³

¹University of Oslo, OSLO, Norway

²Norwegian Medicine Agency, OSLO, Norway

³Westminster University, LONDON, United Kingdom

Chinese medicinal herbs are increasingly used in Europe for contemporary gynaecology. Decoctions of medicinal plant species have a long history in Chinese Medicine and their efficacy is supported with numerous observations. However, preparation methods are shifting from decoctions to concentrated Chinese medicine granules (CCMGs). The production of granules is so far unregulated and non-standardized. Here we test two authentication methods to safeguard purity and falsification of CCMGs. We show the relative performance of traditional barcoding and next generation metabarcoding for species identification in one of the most commonly diagnosed disease patterns in women, endometriosis. First, we selected plant species from contemporary Chinese gynaecology that are frequently used to treat endometriosis. Then, we ordered 100 CCMGs containing these species from distributors and wholesalers across Europe. Of these, 90 reportedly consisted of a single species and are subjected to DNA barcoding and metabarcoding with the nuclear marker *ITS2* to test for adulteration. Ten additional CCMGs consisting of multiple species are investigated with metabarcoding. DNA barcoding is expected to have shortcomings compared to NGS metabarcoding when identifying adulterants in CCMGs. We conclude, that NGS- metabarcoding may be the method of choice to authenticate CCMGs of single, and polyherbal plant mixtures.

Reference :

Mück, F.; Fitzgerald, M.; Odlo Høye, K.; de Boer, H.; Wangenstein, H. Unpublished.

P16

Characterization of inulin-type fructan from *Platycodon grandiflorus* and study on its prebiotic and immunomodulating activity

Berit Smestad Paulsen

School of Pharmacy, OSLO, Norge

Background: *Platycodon grandiflorus* is a plant of widely used in Traditional Chinese Medicine, and polysaccharides are reported to be the main components responsible for its bio-functions.

Methods: In this work, the inulin-type fructan (PGF) was obtained by DEAE anion exchange chromatography of the water extract from *P. grandiflorus*. Characterization was performed with methanolysis, methylation and NMR. In order to study its biofunctions, the prebiotic and immunomodulation properties were assayed.

Findings: The results showed that PGF is a β -(2-1) linked fructan, with terminal glucose, and with a degree of polymerization of 2–10. We found that PGF exhibited good prebiotic activity, as shown by a promotion on six strains of lactobacillus proliferation. Besides, PGF also displayed direct immunomodulation on intestinal epithelial cells, and stimulated the expressions of anti-inflammatory factors.

Conclusion: These results indicated that the inulin from *P. grandiflorus* is a potential natural source of prebiotics, as well as a potential intestinal immunomodulator, which will be valuable for further studies and new applications.

Reference :

Pang,D.; Huang, C.; Chen, M.; Chen, Y.; Fu, Y.; Paulsen, BS.; Rise, F.; Zhang, B.; Chen, Z.; Jia, R.; Li, L.; Song, X.; Feng, B.; Ni, X.; Yin,Z.; Zou, Y. *Molecules*, 2019, In press.

P17

In situ Analysis of Secondary Metabolites in Fungus Infected Apples

Christopher Phippen

Technical University of Denmark, LYNGBY, Denmark

Apples are among the most widely grown fruit in the world and there are many fungi such as *Penicillium expansum*, *Monilinia fructigena*, *Trichothecium roseum* and *Mucor piriformis* which are specialised in growing of apple fruits. The economic importance of apples means much is known about the fungal diseases which can infect and degrade apples. Despite the large amount of research into apple associated fungi, the vast majority of studies are conducted from an agricultural perspective, and very little is known of the secondary metabolites produced by these fungi or their potential effects on human and plant health.

As part of the Center for Microbial Secondary Metabolites (CeMiSt) we have performed *in situ* metabolomics on apples naturally infected with a range of fungal pathogens. This has been coupled to metagenomic analysis and comparison to the isolated fungal species to determine the natural products produced in these complex microbial communities. Using this approach we have begun to determine which secondary metabolites are present in the natural system and we have also discovered several new natural products.

Reference :

Andersen, B.; Smedsgaard, J.; Frisvad, J. C. *J. Agric. Food Chem.*, **2004**, 52 (8), 2421
<https://pubs.acs.org/author/Frisvad%2C+Jens+C>

P18

Chemotaxonomy of a marine proteobacteria, *Pseudoalteromonas* strain S4498

Thomas Isbrandt, Sara Skøtt Paulsen, Lone Gram, Thomas Ostenfeld Larsen

Technical University of Denmark, KGS. LYNGBY, Denmark

Microorganisms are a major source for novel natural products. Bacteria such as actinobacteria have been a major source, and it has recently been shown that also marine Gram-negative bacteria produce many bioactive compounds [1]. The genus *Pseudoalteromonas* encompasses at least 40 different species, many of which produce bioactive compounds [2]. The species within the genus *Pseudoalteromonas* are often grouped based on their pigmentation, with pigmented species often showing a higher level of bioactivity.

In this study, the secondary metabolite profile of a non-pigmented *Pseudoalteromonas* sp. has been characterised. The strain was chosen based on antibacterial activity against *Vibrio anguillarum* and *Staphylococcus aureus*, as well as a secondary metabolite screening, showing the strain to produce a higher variety of compounds compared to related species. Additionally, the strain appeared non-pigmented despite bioinformatics analysis clustering it with other yellow *Pseudoalteromonas* species.

Dereplication indicated the production of a group of quinolone compounds, known as pseudanes, a series of halogenated pyrroles, and an additional number of potentially novel compounds. Both chemical and genetic analysis suggests the strain to be a new species. Further characterisation of the remaining metabolites will be presented, in order to map the secondary metabolite capabilities of the bacterium.

Reference :

1. Schinke, C.; Martins, T.; Queiroz, S. C. N.; Melo, I. S.; Reyes, F. G. R. 2010–2015. *J. Nat. Prod* **2017**, 80, 1215
2. Bowman, J. *Mar. Drugs* **2007**, 5, 220

P19

Phthalide derivatives from *Aspergillus californicus*

Yaojie Guo, Thomas Larsen

Technical University of Denmark, KGS. LYNGBY, Denmark

Aspergillus californicus was first isolated and described forty years ago¹. However, the chemical composition of this

species is less studied. During the search for new antifungal products from filamentous fungi, an extract of *A. californicus* showed anti-candida activity. Dereplication based on LC-DAD-HRMS showed that the active compound and many other compounds are likely unknown. To isolate the antifungal compound as well as other unknown compounds, separation methods such as flash chromatography and prep-HPLC have been used, like spectroscopic techniques such as NMR and HRMS are employed to solve the structure. So far two novel phthalide derivatives, califuranone A1 and A2, together with one known compound emefuranone A2² were discovered. Based on retro-biosynthetic considerations, the three compounds are proposed to originate from the same pathway, where two fungal polyketide synthases (PKSs), one reducing PKS and one nonreducing PKS, are likely involved. Other genes responsible for prenylation, epoxidation, redox reaction, and esterification are likely also needed to build these compounds. All three compounds are predicted to have anti-*Helicobacter pylori* activity due to the presence of the phthalide nucleus³. Linking the underlying gene clusters to these compounds are still ongoing.

Reference :

- (1) Samson, R. A.; Varga, J.; Meijer, M.; Frisvad, J. C. *Stud Mycol* **2011**, 69, 81.
- (2) Saito, T.; Itabashi, T.; Wakana, D.; Takeda, H.; Yaguchi, T.; Kawai, K. I.; Hosoe, T. *Journal of Antibiotics*. **2016**, 69, 89.
- (3) Wang, S.; Zhang, S.; Zhou, T.; Zhan, J. *Bioorganic Med Chem Lett* **2013**, 23, 5806.

P20

Discovery of a novel lichenized algal lineage inspired from chemosystematic and phylogenetic analyses

Maonian Xu¹, Starri Heidmarsson², Elin S Olafsdottir¹

¹University of Iceland, REYKJAVIK, Iceland

²Icelandic Institute of Natural History, AKUREYRI, Iceland

Lichen symbiont interactions (e.g. myco- and photobionts) play a crucial role in the high environmental tolerance of lichens, in which neither symbiont can hardly survive independently. The characterization of fungal-algal association patterns is essential to understand their interactions and to elucidate the symbiotic mechanisms. Lichens are prolific producers of structurally unique secondary metabolites, which are of ecological and taxonomic importance. This study investigated fungal-algal association patterns in Icelandic cetrarioid lichens (168 specimens of 13 cetrarioid taxa) using a multi-locus phylogenetic framework, including fungal nrITS, MCM7, mtSSU and RPB1 and algal nrITS, nrLSU, *rbcL* and mtCOXII data. Lichen metabolite profiles were assessed using UPLC-MS. Our chemical results revealed that *Cetrariella delisei* is the only lichen taxon producing depsides, but not aliphatic lactones, dibenzofurans or depsides. Most Icelandic cetrarioid lichenized fungi were found to be specifically associated to the known *Trebouxia* clade "S" (*T. simplex/suecica* group), while the lichen-forming fungus *Cetrariella delisei* forms a symbiosis with a previously unrecognized *Trebouxia* lineage here provisionally named as the "D" clade. This new *Trebouxia* lineage is supported by Maximum Likelihood and Bayesian phylogenetic analyses using all four included algal loci.

Reference :

Leavitt, S. D.; Kraichak, E.; Nelsen, M.P.; Altermann, S.; Divakar, P.K.; Alors, D.; Esslinger, T.L.; Crespo, A.; Lumbsch, T. *Molecular Ecology* **2015**, 24, 3779

P21

How homoplastic is the production of usnic acids? a re-appraisal on enantiomer distribution pattern and its ecological implications

Maonian Xu¹, Starri Heidmarsson², Elin S Olafsdottir¹

¹University of Iceland, REYKJAVIK, Iceland

²Icelandic Institute of Natural History, AKUREYRI, Iceland

Usnic acid (UA) is one of the most ubiquitous lichen compounds present in lichen cortex. It could constitute as high as 8% dry thallus weight. It has two enantiomers with marked antibiotic activity, where (+)-UA tends to be a better antimicrobial agent, and (-)-UA is a more potent phytotoxin. Despite such high contents and pronounced bioactivities, the distribution of isomers has not been explored in a phylogenetic context. The current study aimed to develop an analytical method characterizing the content and ratio of UA isomers in Parmeliaceae lichens, and to explore the phylogenetic signal for UA isomer production. UA was fractionated using a preparative HPLC method, and isomeric ratio was determined using a chiral HPLC method. Phylogenetic relationship was estimated using a multi-loci analysis. Our results show that UA could account for 1.65-4.37 mg/g d.w. The production of UA isomers tend to be intraspecifically homogeneous with one isomer as the predominant and the other minor or absent. A strong phylogenetic signal was found using Icelandic UA-producing lichen taxa: terricolous taxa in the cetrarioid group and the allied genus *Alectoria* mainly produce (-)-usnic acid, and the epiphytic sister genus *Usnea* only produces (+)-usnic acid.

Reference :

Kinoshita, Y.; Yamamoto, Y.; Yoshimura, I.; Kurokawa, T.; Huneck, S. *J. Hattori Bot. Lab.* **1997**, 83, 173

Synthesis of antioxidant chitosan conjugates using tert-butyldimethylsilyl (TBDMS) protection

Vivien Nagy, Priyanka Sahariah, Martha Á. Hjálmsdóttir, Már Másson
Háskóli Íslands, REYKJAVÍK, Iceland

Chitosan possesses antimicrobial, antitumor, hemostatic, analgesic, film-forming and mucoadhesive activities and is non-toxic, biodegradable and biocompatible. However, good antimicrobial activity is only achieved under pH 6.5 and chitosan has poor aqueous solubility above this. Chemical modification can be used to overcome these issues.

The aim of the current work was to develop a new and efficient synthesis procedure for conjugating antioxidants to chitosan, based on the use of tertbutyldimethylsilyl (TBDMS) protection. The protected antioxidants were converted to their corresponding acid chlorides and reacted with TBDMS protected chitosan in dichloromethane to form amide linkages. The TBDMS protection was then removed to obtain water soluble antioxidant chitosan conjugates.

The structure and degree of substitution (DS) of the products were confirmed by H-NMR spectroscopy. With the new method, up to DS=53% was obtained. The antibacterial activity against *S.aureus* and *E.coli*. All conjugates were found to be bactericidal. While chitosan possessed no antioxidant activity in the tested concentrations, the conjugates exhibited increasing DPPH scavenging activity with increasing DS.

A new method was developed for the synthesis of antioxidant chitosan conjugates using the DOE approach. The conjugates exhibited significant increase in the DPPH scavenging activity compared to chitosan (up to 433 fold increase in IC₅₀).

Reference :

Sahariah, P.; Hjálmsdóttir, M. Á.; Másson, M. Antimicrobial properties of chitosan and chitosan derivatives. *Marine Glycobiology: Principles and Applications*. S.-K. Kim 2017, CRC Press: 345-369.

Sahariah, P.; Gaware, V. S.; Lieder, R.; Jónsdóttir, S.; Hjálmsdóttir, M. Á.; Sigurjonsson, O. E.; Másson, M. The effect of substituent, degree of acetylation and positioning of the cationic charge on the antibacterial activity of quaternary chitosan derivatives. *Marine drugs* 2014, 12 (8) 4635-4658

Hu, Q.; Luo, Y. Polyphenol-chitosan conjugates: Synthesis, characterization, and applications. *Carbohydrate Polymers* 2016 151 624-639

Lee, D. S.; Woo, J. Y.; Ahn, C. B.; Je, J. Y. Chitosan-hydroxycinnamic acid conjugates: preparation, antioxidant and antimicrobial activity. *Food Chem* 2014 148 97-104

Pathway discovery and biotechnological production of bioactive diterpenoids: a route to sustainable manufacturing of plant-derived pharmaceuticals

Dan Luo¹, Victor Forman¹, Allison Heskies¹, Johan Andersen-Ranberg¹, Kyle Lauersen², Dan Stærk¹, Birger Møller¹, Irini Pateraki¹

¹University of Copenhagen, FREDERIKSBERG C, Danmark

²Bielefeld University, BIELEFELD, Germany

Plants produce more than 11,000 different diterpenoids and they display a wide range of biological activities. Some are used directly as therapeutics for treatment of human diseases, such as the cyclic AMP booster forskolin, anti-cancer drugs paclitaxel and ingenol mebutate. Others serve as lead compounds in drug discovery and have been the focus of many medicinal chemistry and therapeutic studies. However, most bioactive diterpenoids were isolated from non-cultivated medicinal plants or from endangered or red listed species. The lack of a reliable and environmentally benign and stable supply chain thus remains a major obstacle when the desire arises to develop a specific diterpenoid into a medicinal drug. Using forskolin and ingenol mebutate as examples, we provide an account on how production of these plant-derived pharmaceuticals may shift from field acquisition to sustainable manufacturing. To achieve this, knowledge of the enzymes involved in the biosynthetic pathway is essential. Through the strategy consisting of metabolite profiling, transcriptome analysis and enzyme characterization, we have managed to elucidate biosynthetic pathways of several bioactive diterpenoids including forskolin, jolkinol C and vitexilactones. Subsequently, reconstitution of elucidated pathways in microbial hosts like yeast or photosynthetic microalgae offers the sustainable production of targeted plant diterpenoids.

Reference :

(1) Luo, D.; Callari, R.; Hamberger, B.; Wubshet, S. G.; Nielsen, M. T.; Andersen-Ranberg, J.; Hallström, B. M.; Cozzi, F.; Heider, H.; Møller, B. L.; Staerk, D.; Hamberger, B. *Proc. Natl. Acad. Sci. U. S. A.* 2016, 113, E5082.

(2) Pateraki, I.; Andersen-Ranberg, J.; Jensen, N. B.; Wubshet, S. G.; Heskies, A. M.; Forman, V.; Hallstrom, B.; Hamberger, B.; Motawia, M. S.; Olsen, C. E.; Staerk, D.; Hansen, J.; Møller, B. L.; Hamberger, B.; *eLife* 2017, 6, e23001.

(3) Heskies, A. M.; Sundram, T. C. M.; Boughton, B. A.; Jensen, N. B.; Hansen, N. L.; Crocoll, C.; Cozzi, F.; Rasmussen, S.; Hamberger, B.; Hamberger, B.; Staerk, D.; Møller, B. L.; Pateraki, I. *Plant J.* 2018, 93, 943.

P24

A new acetylcholinesterase inhibitor from Icelandic bryozoan *Flustra foliacea*

Natalia Magdalena Kowal, Xiaxia Di
University of Iceland, REYKJAVIK, Iceland

Background: Acetylcholinesterase (AChE) inhibitors are the main strategy in symptomatic treatment of Alzheimer's disease. Currently three drugs are on the market, galantamine a natural product, rivastigmine a derivative of natural product and synthetic donepezil. However, new drugs with better activity/adverse effects profile are needed. Bryozoan *Flustra foliacea* produce a range of indole alkaloids which have shown weak antibiotic, muscle-relaxant and cytotoxic properties, however most of them has not been tested in vitro. Many of them possess physostigmine scaffold, well-known AChE inhibitor, therefore they might be attractive drug leads for the treatment of Alzheimer's disease.

Methods: *In vitro* AChE inhibitory activity was studied using the colorimetric method of Ellman using AChE enzyme from *Electrophorus electricus*. Colorimetric detection performed at 405 nm.

Results: Twenty-six compounds isolated from Icelandic bryozoan *Flustra foliacea* were screened at a 100 μ M concentration against AChE. Two of them, deformylflustrabromine and flustramine I, showed inhibition close to 50% and one, flustramine Q¹, 92%. For flustramine Q an IC₅₀ value of 9.6 μ M was determined.

Conclusions: Marine natural products including brominated indole alkaloids from *Flustra foliacea* could serve as a source of active compounds with potential towards central nervous system diseases, e.g. Alzheimer's disease.

Reference :

¹ Di X. (2019). *Searching for immunomodulatory compounds from Icelandic marine invertebrates* (Doctoral dissertation, University of Iceland, Reykjavik, Iceland)

P25

Effect of faltarindiol purified from carrots on colon cancer cells

Camilla Andersen¹, Emma Pipó-Ollé¹, Anders Walther¹, Richard Cowie², Martin Hedegaard¹, Eva Arnspang¹

¹University of Southern Denmark, ODENSE, Denmark

²The Maersk Mc-Kinney Moller Institute, University of Southern Denmark, ODENSE, Denmark

Faltarindiol (FaDOH) are a cytotoxic and anti-inflammatory polyacetylenic oxylipins found in food plants of the carrot family (Apiaceae). In this study, the effect on selected biomarkers in mammalian cell culture relevant for colon cancer treated with FaDOH was studied. Furthermore, the effect of delivering the FaDOH in nanoparticles was investigated. Preliminary data indicate effect on genes involved in inflammation, we used quantitative PCR to look at the expression level of TNF α , and IL8, COX-1, COX-2, PPAR γ 2. Additionally, we have studied the FaDOH inside cell using fluorescence microscopy, Raman imaging and CARS. This study therefore gives insight into the anti-cancer properties of FaDOH.

P26

Fluorinated secondary metabolites from halotolerant endophytic fungi

Louise Kjaerulff, Mahdiehalsadat Hosseynimoghadam, Kenneth Thermann Kongstad
University of Copenhagen, COPENHAGEN, Denmark

Background Fluorine is the most abundant halogen on Earth and the 13th most abundant element in the Earth's crust. Many pharmaceuticals contain fluorine and the element has an increasing role in drug development, with 30% of new approved small-molecule drugs containing fluorine [1]. However, fluorinated organic molecules are extremely rare in nature, and only a handful of fluorine-containing natural products have been reported.

Methods Laboratory cultivations of fungi with various salts combined with ¹⁹F NMR-guided isolation, HPLC-PDA-HRMS, and 2D NMR spectroscopy were used to investigate inducible production of fluorinated compounds.

Findings A series of halotolerant, endophytic filamentous fungi were isolated from plants growing in arid soil in the deserts of northern Iran. Chemical profiling of cultivations with various salts allowed us to target both fluorinated molecules and other natural products induced with these cultivation conditions. Several new polyketide- and terpene-derived metabolites were discovered, including a family of complex fluorinated secondary metabolites from *Phomopsis azadirachtae*.

Conclusion The results from this study show that production of fluorinated natural products is inducible under laboratory conditions in halotolerant fungi, adding unique natural products to a very small group of fluorinated natural products.

Reference :

[1] Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Xhu, W.; Aceña, J. ; Soloshonok, V. A.; Izawa, K.; Liu, H. *Chem. Rev.* **2016**, *116*, 422.

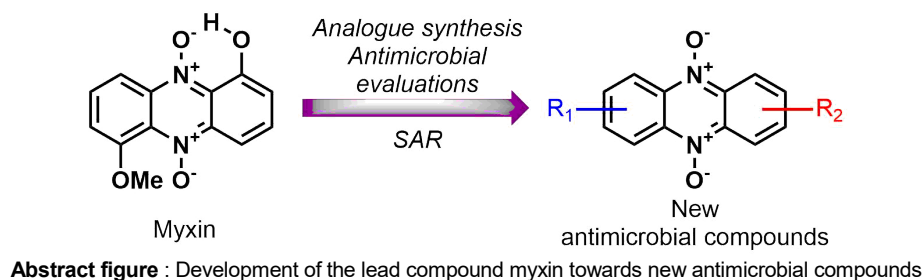
Synthesis and antimicrobial evaluations of new phenazine 5,10-dioxides derived from myxin

Guðjón Trausti Skúlason¹, Alexander Høgmoen Åstrand², Pål Rongved² and Elvar Örn Viktorsson¹

¹University of Iceland, REYKJAVÍK, Iceland

²University of Oslo, OSLO, Norge

Phenazines constitute a large class of secondary metabolites from bacteria. The natural product myxin (1-hydroxy-6-methoxyphenazine 5,10-dioxide) is well known for potent antimicrobial activity and its broad spectrum against Gram-positive bacteria, Gram-negative bacteria and fungi.¹ Myxin has also shown sub-micromolar IC₅₀ values causing apoptotic cell death in human acute myeloid leukemia (AML) cancer cells.² This poster will highlight the synthetic work of phenazine 5,10-dioxide myxin derivatives and their in vitro antimicrobial activities.



Reference :

Antimicrob. Agents. Ch, 1970, **10**, 46.

Bioorg. Med. Chem, 2017, **25**, 2285.

NNPC CONFERENCE AUTHORS INDEX

A

Ahl, Louise Isager L11
Ahring, Philip Kiær L09, L27
Anam, Syariful L20
Andersen, Bjarke P01
Andersen, Camilla P25
Andersen-Ranberg, Johan P23
Antonelli, Alexandre P13
Arnspang, Eva L26, P25

B

B. Wolff, Peter L18
Baatrup, Gunnar L26
Backlund, Anders L13
Bak, Søren L14, P09
Balle, Thomas L09, L27
Barnes, Christopher L11, P13
Bonde, Charlotte Smith L01
Bornancin, Louis L01
Bourgau, Frédéric P05

C

Canales, Nataly Allasi P13
Cardenas, Pablo D. P09
Cárdenas, Paco L17
Chebib, Mary L09, L27
Christopoulos, Panagiotis L25
Clark, Chase L02
Cornett, Claus P13
Corthay, Alexandre L25
Cos, Paul IL02
Costa, Maria L02
Cowie, Richard P25
Cronberg, Nils P10

D

De Boer, Hugo J. P12, P15
Deniz, Tasdemir IL01
Di, Xiaxia P24

E

Einarsson, Hjorleifur L05
Eiriksson, Finnur Freyr L07, P10
El-Houri, Rime L26
Erngren, Ida L17
Eythorsdottir, Arnheidur L05

F

Fallarero, Adyary P02, P03, P04
Fitzgerald, Martin P15
Fomsgaard, Inge S. L24
Forman, Victor P23
Fretté, Xavier P01
Frisvad, Jens C. L18

G

Gede Indraningrat, Anak Agung L17
Gerwick, William P11
Gilbert-Girard, Shella P03
Gottfredsen, Charlotte Held L16, L18
Grace, Olwen M. L11
Gram, Lone P18
Guðmundsson, Guðmundur Hrafn IL03
Guo, Yaojie P19

H

Haglöf, Jakob L17
Hansen, Tobias Nikolaj Gress P13
Harvey, Benjamin S L27
Hauser, Thure P09
Hedegaard, Martin L26, P25
Heidmarsson, Starri L12, P20, P21
Heskes, Allison P23
Hjálmarsson, Martha L23, P22
Høgmoen Åstrand, Alexander P27
Hoof, Jakob Blæsbjerg L16
Hosseynimoghadam, Mahdiehalsadat L20, P26

I

Indurthi, Dinesh C. L27
Inngjerdningen, Kari L25, P11
Isbrandt, Thomas P18

J

Jónsdóttir, Sigríður L23
Jørgensen, Bodil L11

K

Kaupilla, Jussi P02
Kjærulff, Louise L20, P26
Klejnstrup, Marie Louise L16
Kobaek-Larsen, Morten L26
Kongstad, Kenneth L20
Kongstad, Kenneth Thermann P26
Kowal, Natalia Magdalena L09, L27, P24
Kristjánsdóttir, Ingibjörg L23
Kryger, Per L24

L

Larsen, Thomas Ostenfeld L03, L16, L18, P18, P19
Lauersen, Kyle P23
Liao, Vivian W Y L27
Loncarevic, Isidora P10
Lu, Yi L07
Luo, Dan P23

M

Másson, Már L21, L23, P14, P22
Mejer, Helena L01
Miguel, Sissi P05
Møller, Birger P23

Mortensen, Uffe Hasbro L16, L18
Moussavi, Nastaran P08
Mück, Felciitas P15
Murmah, Toke P01
Murphy, Brian L02

N

Nagy, Vivien P22
Nesbitt, Mark P13
Notabi, Martine L26

O

O'Connor, Susan M L27
Oddsson, Sebastian L09
Odlo Høye, Kristin P15
Omarsdóttir, Sessella L02, L05, L12

Ó

Ólafsdóttir, Elín Soffía L09, L12, L27, P20, P21

P

Pateraki, Irini P23
Paulsen, Berit Smestad P16
Pedersen, Hans Albert P07
Pedersen, Henriette L. L11
Peltonen, Jouko P02
Petersen, Malene J. P06
Phippen, Christopher P17
Pipó-Ollé, Emma L26, P25
Porskjaer Christensen, Lars L26
Ptak, Signe P01

R

R. Andersen, Mikael L18
Rathinam, Sankar L23
Ravikumar, Kaliamoorthy P12
Reigada, Inés P04
Rongved, Pål P27
Rønsted, Nina L10, L11, P13
Rosqvist, Emil P02
Rysbjerg, Karen P09

S

Sahariah, Priyanka L23, P22
San Martin Galindo, Paola P02
Sanchez, Laura L02
Savijoki, Kirs P03, P04
Seethapathy, Gopalakrishnan S. P12
Semple, Susan L06, P07
Sigurbjörnsson, Kristinn Páll P14
Simonsen, Henrik Toft L01, L07, P05
Sipkema, Detmer L17
Skøtt Paulsen, Sara P18
Skúlason, Guðjón Trausti P27
Solodova, Svetlana P14
Sorensen, John L15
Staerk, Dan L06, L20, P07
Stærk, Dan P06, P23

Steffen, KarinL17
Subko, KarolinaL18

T

Thamsborg, Stig MilanL01
Theobald, Sebastian.....L18
Thi Dinh, NhungP07

V

Vidkjær, Nanna HjortL24
Viktorsson, Elvar ÖrnL04, P27
Volpatto Marques, RaïssaP05

W

Walker, KimP13
Walther, AndersL26, P25
Wang, XinhuiL16
Wangensteen, Helle P11, P12, P15
Ward, JaneL24
Willats, William G. T.....L11
Williams, Andrew RichardL01
Wold, ChristianL25, P11

X

Xu, Maonian L12, P20, P21

Y

Yli-Kauhaluoma, Jari P03, P04

Z

Zhao, YongL06

Þ

Þórleifsdóttir, SigrúnL08
Þorsteinsdóttir, MargrétL07, P10

Ö

Örvar, BjörnL19