



*Perspectives from the SUBMARINER Blue Biotechnology Cooperation Event  
Kiel, Germany | 9–10 May 2012*

# Blue Biotechnology in the Baltic Sea Region: New Strategies and Future Perspectives



Part-financed by the European Union  
(European Regional Development Fund)



Baltic Sea Region  
Programme 2007–2013

[www.submariner-project.eu](http://www.submariner-project.eu)

## 6 State-of-the-Art and Future Perspectives

- 7 Marine Biotechnology opportunities and challenges – Are we realising the vision and strategy for Europe?** | Jan-Bart Calewaert
- 8 Blue Biotechnology all around the Baltic Sea** | Jutta Wiese
- 10 Six priorities proposed for marine biotechnology in Denmark** | Torger Børresen
- 12 Trends of biotechnology in Latvia** | Juris Vanags
- 13 MAREX: Exploring marine resources for bioactive compounds – From discovery to sustainable production and industrial applications** | Päivi Tammela, Paula Kiuru, Tiina Lipiäinen, Jari Yli-Kauhaluoma & Heikki Vuorela
- 15 Blue Biotechnology for sustainable innovations – a Blue Biotech project in the Kattegat and Skagerrak**
- 16 Blue Biotechnology in Germany – the Kiel Centre for Marine Natural Products KiWiZ** | Johannes F. Imhoff
- 18 *Spila Spirulina* microalgae applications for human food, health and animal feed** | Vytas Rimkus
- 20 Blue Biotechnology Research Centres in Poland** | Alicja Kosakowska & Adam Żak

## 22 Science meets Industry

- 23 Biotechnological advances in discovery and biosynthesis of bioactive secondary metabolites in marine microalgae** | Allan D. Cembella
- 25 Knowledge capture mechanism studies as a tool to facilitate European Blue Biotech studies** | Daniel Pardo, Sophie Arnaud-Haond, Jesus M. Arrieta & Antoine Schoen
- 26 Marine functional genomics** | Antje Gardebrecht
- 27 Fungi associated to mediterranean seagrasses and algae: diversity, ecological role and potential exploitation** | Giovanna Cristina Varese, L. Panno & G. Gnani, A. Kramer & A. Labes
- 29 Knowledge transfer from environmental genomic science to marine biotechnology – big data, big challenges** | Johanna B. Wesnigk
- 31 Analysis of cell powder from *in vitro* cultured fish cells** | Marina Gebert
- 32 Production of omega-3 fatty acids by Baltic Sea algae** | Kristian Spilling, Jukka Seppälä, Niklas Virkkala, Susanna Nenonen, Elina Salo, Katarina Natunen, Timo Tamminen, Dagmar Enss & Heiko Rischer
- 33 Healthy appetite for Algae: Milk products containing algae** | Marie Shrestha
- 34 From sustainably cultivated seaweed to certified organic cosmetics – The brand “Oceanwell” as a success story for a value-added chain originating from ecologically farmed marine resources** | Inez Linke
- 35 Sulfated polysaccharides of *Delesseria sanguinea* from the artificial reef Nienhagen** | Susanne Alban, Niels Grünewald, Juliane Grimm & Inken Groth
- 37 From marine biodiversity to innovative drugs. The experience of PharmaMar** | Fernando de la Calle
- 39 Marine microorganisms – a promising source for novel therapeutics** | Sabine Mundt, Wolf-Dieter Jülich, Ulrike Lindequist & Gerold Lukowski
- 41 AAC – Algae Against Cancer: Preliminary data of a national research project** | Marion Zenthoefer, L. Piker, M. Peipp, M. Gramatzki, U. Geißen, H. Kalthoff, Y. Weniger, S. Alban, S. Hennig, M. Fuhrmann, F. Huber & R. Kirchhöfer
- 42 Integrated analytical approaches towards marine natural products discovery** | Thomas Ostfeld Larsen, Maria Månsson, Kristian Fog Nielsen, Charlotte Held Gotfredsen, Lone Gram, Per Juel Hansen
- 43 Aquapharm – Application of Blue Biotechnology for the discovery of novel pharmaceuticals** | Andrew Mearns Spragg
- 45 Genome based methods for the exploration of natural products from marine fungi for the treatment of cancer** | Antje Labes

### CONFERENCE ORGANISERS



GEOMAR | Helmholtz Centre for Ocean Research Kiel  
Dr. Jutta Wiese  
Düsternbrooker Weg 20  
DE – 24105 Kiel  
jwiese@geomar.de



Norgenta GmbH  
Dr. Imke Schneemann  
Wissenschaftspark Kiel  
Fraunhoferstraße 2-4  
DE – 24118 Kiel  
imke.schneemann@norgenta.de

North German  
Life Science Agency

### SUBMARINER LEAD PARTNER



The Maritime Institute in Gdańsk  
Joanna Przedzrymirska  
Długi Targ 41/42  
PL – 80-830 Gdańsk  
joaprz@im.gda.pl

### EXTERNAL PROJECT COORDINATION OFFICE



s.Pro – sustainable projects GmbH  
Angela Schultz-Zehden  
Rheinstraße 34  
DE – 12161 Berlin  
asz@sustainable-projects.eu

### EDITORIAL, LAYOUT AND DESIGN

s.Pro – sustainable projects GmbH  
Tommi Vollmann

### DISCLAIMER

This publication was produced with the assistance of the European Union (European Regional Development Fund). Its content is the sole responsibility of the authors and can in no ways be taken to reflect the views of the European Union.

### Find more on our website

More impressions from the Blue Biotechnology Cooperation Event as well as presentations can be found on the SUBMARINER project website:

[www.submariner-project.eu](http://www.submariner-project.eu)





*SUBMARINER hosted a Blue Biotechnology Cooperation Event in Kiel, Germany on 9 and 10 May 2012.*

*More than 150 experts from science, policy and business discussed new strategies and future perspectives of Blue Biotechnology in the Baltic Sea Region.*

## Foreword



**Joanna Przedzimirska**  
The Maritime Institute in Gdańsk  
SUBMARINER Lead Partner

Blue Biotechnology has the potential to offer answers to global challenges related to human health, food demands, and environmental concerns. It is still a young discipline that currently develops its contours. But the global market for products, technology and services based on marine resources continuously increases – a fact that requires effective networking and adopting a solid position to persist within this very dynamic surrounding.

The Marine Board of the European Science Foundation (ESF) rates the global market in this segment to be 2.8 billion €, with annual growth rates of 12 %. A background report of the EU's Blue Growth initiative predicts the Baltic Sea Region to play a major role in Blue Biotechnology in the future.

SUBMARINER is for the first time comprehensively assessing the economic feasibility, the regional applicability and the environmental impacts of a number of new marine uses in the Baltic Sea Region. With a view to its enormous development potential, we selected Blue Biotechnology as one of the topics for this assessment. The perspectives assembled in this publication are thus an important input to the policy recommendations for furthering beneficial marine uses that we are developing within the project. They are based on the presentations held at the SUBMARINER Blue Biotechnology in Kiel, Germany in May 2012.

Kiel was the obvious place to host this Cooperation Event as some of the Baltic Sea Region's key research institutions are located there. What is more, Schleswig-Holstein is currently developing a comprehensive regional development strategy for Blue Biotechnology as part of the SUBMARINER project.

After an algae conference in September 2011, the Blue Biotechnology conference was the second in a series of SUBMARINER Cooperation Events. In collaboration with the Aquabest project, SUBMARINER is now co-organising the Åland Aquaculture Week to be held in October 2012. This event will provide the opportunity to discuss ways towards a sustainable fish aquaculture and the potential of mussel farming in the Baltic Sea Region.

### Key messages for the Baltic Sea Region

Can the Baltic Sea Region be a model region for Blue Biotechnology? And which steps have to be taken to strengthen the region's competitiveness in this field? These were the leading questions of the Cooperation Event's concluding panel discussion. These are some of the recommendations that the panelists came up with:

- Develop national Blue Biotechnology strategies in cooperation with other Baltic Sea Region states and aligned with the EU level,
- Formulate clear ambitions for the Baltic Sea Region in the field of Blue Biotechnology and focus on strengths and market needs,
- Make sure that Blue Biotechnology is "truly blue" and sustainable, i.e. to the benefit of the sea,
- Improve the modalities of technology transfer to achieve better exploitation of scientific results and patents,
- Make better use of existing support technologies and platforms and strengthen application-oriented approaches



**The Panelists:** Jan-Bart Calewaert (Marine Board – ESF), Steffen Lüsse (Ministry of Economic Affairs, Employment, Transport and Technology of Land Schleswig-Holstein), Juris Vanags (Association of Biotechnology of Latvia), Allan Cembella (Alfred Wegener Institute) Fernando de la Calle (PharmaMar), Torger Børresen (DTU Food)

# State-of-the-Art and Future Perspectives of Blue Biotechnology



At the European level, Blue Biotechnology is seen as an important contribution to addressing grand challenges of the 21<sup>st</sup> century and to fulfilling the Europe 2020 strategy. Jan-Bart Calewaert from the Marine Board of the European Science Foundation presents some of the key policy documents on the European level, such as the Copenhagen Declaration for a Bioeconomy in Action and the Marine Board's Position Paper on Marine Biotechnology.



On the Baltic Sea Region level, an overarching strategy for Blue Biotechnology does not yet exist. In her contribution, Jutta Wiese (GEOMAR | Helmholtz Centre for Ocean Research Kiel) shows that such a Baltic Sea Region strategy is needed to realise the visions and recommendations that the SUBMARINER project has developed based on its assessment of the state-of-the-art of Blue Biotechnology around the Baltic Sea.

Denmark is currently the only Baltic Sea Region country that has a national Blue Biotech strategy in place. In his article, Torger Børresen (DTU Food) describes the six priorities of the Danish government's 2010 report on the development of marine biotechnology in Denmark.

In Germany, a regional Masterplan Marine Biotechnology for the state Schleswig-Holstein is being developed as part of the "Sea our Future" initiative within the SUBMARINER project. Focusing on the Kiel Centre for Marine Natural Products, Johannes F. Imhoff (GEOMAR | Helmholtz Centre for Ocean Research Kiel) introduces the Northern German research institutions active in the field.



The key drivers for furthering Blue Biotechnology in the Baltic Sea Region are its strong research institutions. Ongoing Blue Biotech projects from Finland and Poland are presented by Alicja Kosakowska and Adam Żak (Institute of Oceanology, Polish Academy of Sciences) as well as Päivi Tammela (University of Helsinki).

A showcase from Lithuania demonstrates how Blue Biotechnology can be turned into profitable business: Vytas Rimkus presents the microalgae applications for human food, health and animal feed that his company SPILA is involved in.

## Marine Biotechnology opportunities and challenges: Are we realising the vision and strategy for Europe?

Jan-Bart Calewaert | Marine Board-ESF, Belgium

The interest of the scientific community, and to a lesser extent of industry, in marine biotechnology has grown rapidly in the past decade owing to a recognition of the sheer scale of opportunity presented by the largely unexplored and unexploited biodiversity of our seas and oceans and the need to meet growing demands that cannot be satisfied from terrestrial sources alone. Several publications (e.g. from Marine Board-ESF, European Commission CWG and EU-US Task Force on Biotechnology) have highlighted the importance of marine biotechnology and its potential to make a significant contribution to sustainable development on all fronts, including social, economic and environmental. However, it is only since recently that a concerted effort is being made in Europe to develop an initiative that seeks to take action on many of the issues identified, to improve the coordination of marine biotechnology research, raise its profile, and contribute to the establishment of the Knowledge Based BioEconomy (KBBE).

This presentation sketched key policy developments of European marine biotechnology in the last decade, highlighted areas of progress and addressed future perspectives. It presented some of the main opportunities and challenges identified by strategic exercises and assessed where we are in realising the vision and strategy for Europe proposed in Marine Board Position Paper 15 on Marine Biotechnology. Finally, the presentation highlighted some of the outcomes of the FP7 CSA MARINEBIOTECH project ([www.marinebiotech.eu](http://www.marinebiotech.eu)), in particular the preliminary results of the mapping of European Marine Biotechnology Research landscape being conducted by Marine Board-ESF.

### References

Arrieta J, Arnaud-Haond S, Duarte, C. Marine reserves Special Feature. What lies underneath: Conserving the oceans' genetic resources. Proc. Natl. Acad. Sci. U. S. A. 2010 Early Edition Special Feature: Perspective; 1–7.

Bouchet P. The magnitude of marine biodiversity. In: Duarte C, editor. The exploration of marine biodiversity: scientific and technological challenges. Bilbao: Fundación BBVA; 2006. p. 31–62.

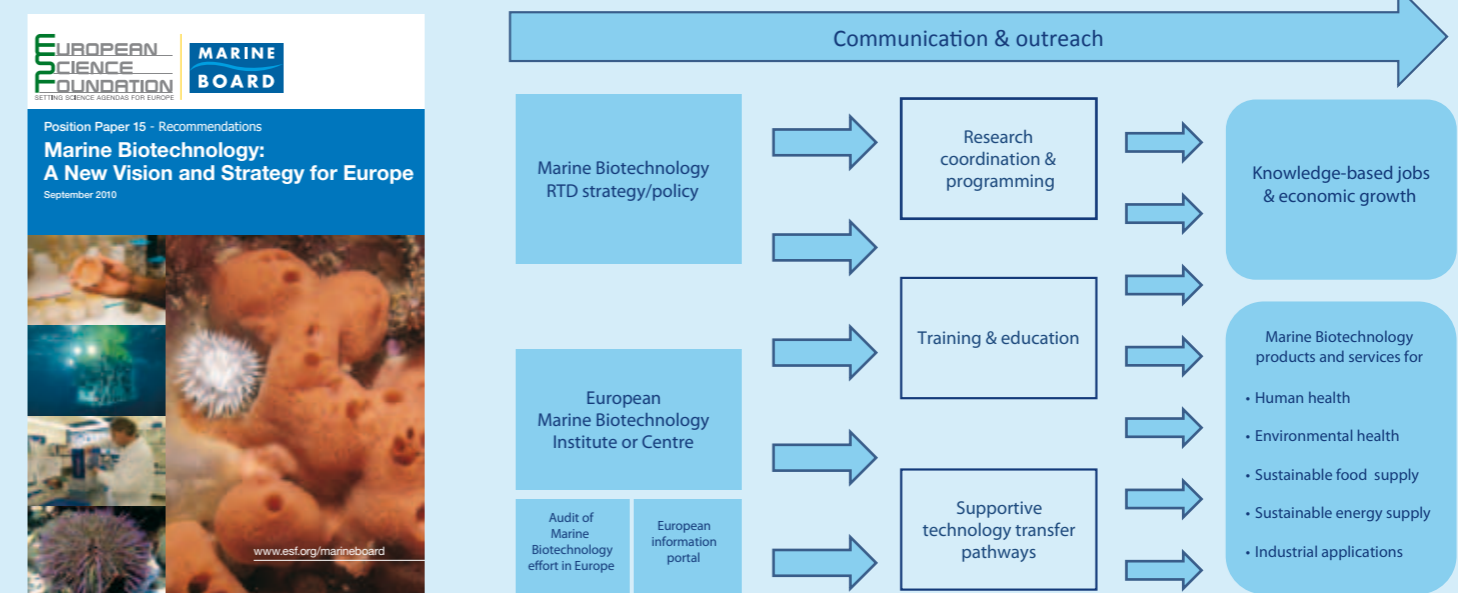
Halvorson H, Alexis M, Burgess G, Coimbra J, le Gal Y, Grealy M, Gutnick D, Höfle M, Smolenicka Z, Walther B, Wenne R, Olafsen JA. Marine Biotechnology: A European Strategy for Marine Biotechnology. Marine Board Position Paper 4. Strasbourg: Marine Board-ESF; 2001.

Heip et al., Marine Biodiversity and Ecosystem Functioning, 2009. ISSN 2009–2539.

National Research Council. Marine Biotechnology in the Twenty-first Century: Problems, Promise, and Products. Washington D.C.: National Academy Press; 1999.

Querellou J, Børresen T, Boyen C, Dobson A, Höfle M, Ianora A, Jaspars M, Kijjoo A, Olafsen J, Rigos G, Compère C, Magot M, Olsen J, Potin P, Volckaert F, Wijffels R. Marine Biotechnology: a new Vision and Strategy for Europe. Marine Board Position Paper 15. In: Calewaert JB, McDonough N, editors. Oostende: Marine Board-ESF; 2010.

### Overview of recommendations from the Marine Board-ESF's position paper on Marine Biotechnology



Identity & profile of European Marine Biotechnology research

## Blue Biotechnology all around the Baltic Sea

Jutta Wiese | GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

The SUBMARINER project seeks to turn the Baltic Sea into a model region by fostering sustainable economic development and improved environmental conditions through new maritime products and technologies. In this project the Kieler Wirkstoff-Zentrum (Centre of Marine Natural Products) at GEOMAR focuses on aspects of Blue Biotechnology.<sup>1</sup> It is responsible for the chapter on Blue Biotechnology in the SUBMARINER Compendium, a comprehensive inventory of innovative uses of Baltic marine resources.

According to the Internal Co-ordination Group for Biotechnology (ICGB) Blue Biotechnology has a considerable potential to address global challenges in population health, food security, industry and environmental sustainability as well as protecting and preserving marine resources for future generations.<sup>2</sup> The exploration of marine microorganisms (bacteria, fungi, microalgae) and macroorganisms, such as algae or mussels, which are largely untapped, is a promising tool to find solutions for these challenges.

Examples for solutions provided by Blue Biotechnology are:

- **Strong need for the discovery of therapeutic agents:** providing new drugs for the treatment of metabolic diseases (diabetes), inflammatory diseases, neurological diseases (dementia), cardiovascular diseases (stroke), and diseases caused by antibiotic resistant pathogens, etc.
- **Strong need for new products with environmental friendly properties in comparison to known products:** providing new compounds used in plant protection, new feed for the cultivation of marine organisms in aquaculture, new products for the treatment of diseases of marine organisms, new anti-fouling paints, etc.

- **Strong need for new technologies to be used in the improvement of the environmental conditions:** providing new products for the reduction of nutrients in marine habitats, bioremediation, environmental monitoring, etc.

Furthermore, Blue Biotechnology provides a huge economic value. According to the Marine Board of the European Science Foundation (ESF) the market in this segment is rated to be 2.8 billion € with an annual growth potential of 12% assuming a cooperation of industry and science.<sup>3</sup>

### Assessing Blue Biotechnology in the Baltic Sea Region

The SUBMARINER Compendium aims at assessing the state-of-the-art and perspectives of Blue Biotechnology of all countries around the Baltic Sea, to evaluate further applications and to define technical requirements taking into account environmental aspects, legal regulations and economic aspects. This assessment will characterise the future potential of marine resources for Blue Biotechnology and will work out major obstacles to the further development of this field.

Promising scientific approaches and case studies of companies working in the discovery and/or economical exploitation of resources from the Baltic Sea are present in all countries, which are covered by the study, i.e. Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, and Sweden. Among these resources are raw materials, valuable ingredients such as bioactive compounds, fatty acids, enzymes, biopolymers as well as genetic resources for biotechnological applications in pharmaceutical industry, medical products, human diet, animal feed, cosmetic, wellness sector, bioremediation and others (Figure 1).



*On all shores of the Baltic Sea, the potential of Blue Biotechnology has been recognized.*

All activities in this field aim to enhance the awareness in the public and in the scientific community, to improve human and environmental health as well as to increase economic benefits.

The potential of Blue Biotechnology of providing benefits for academia, companies, society and policy has been recognized and becomes visible by the following examples:

- **Europe:** Report “Blue Growth - Scenarios and drivers for Sustainable Growth from the Oceans, Seas and Coasts” on behalf of the DG Mare of the European Commission (2012)<sup>4</sup>
- **Denmark:** Report „The Ocean – an unexploited resource” by the Ministry of Food, Agriculture and Fisheries Denmark (2010)
- **Estonia:** Report on „Estonian biotechnological programme, a feasibility study” (2010)
- **Finland:** EU project MAREX (2010–2014)
- **Germany:** Initiative “Zukunft Meer - Sea our Future” by the Ministry of Science, Economic affairs and Transport Schleswig-Holstein (2005); “Kieler Wirkstoff-Zentrum”; “Masterplan Marine Biotechnology Schleswig-Holstein” (in progress); EU projects Marine Fungi and MicroB3
- **Latvia:** Establishment of the Latvian Biotechnology Association (2006)
- **Lithuania:** Studies on microalgae – diversity and potential for biotechnology (in progress)
- **Poland:** EU project MAREX (2010–2014)
- **Sweden:** INTERREG IV A project Blue Biotechnology for sustainable innovations in the region Öresund-Kattegat-Skagerrak (cooperation with Norway, 2011–2013)

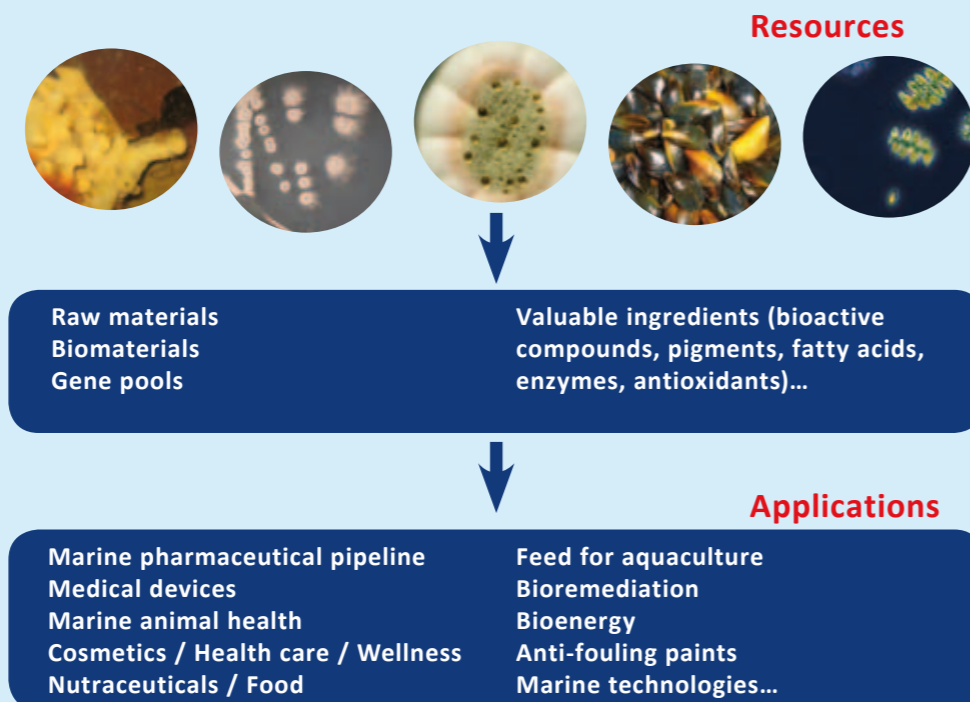
The first results from the SWOT (Strengths, Weakness, Opportunities, Threats) analysis carried out for the SUBMARINER Compendium clearly demonstrate the high potential of Blue Biotechnology for the Baltic Sea Region. Especially the development of a strategy for the implementation of promising fields of Blue Biotechnology in the Baltic Sea Region would help in the realisation of visions such as:

- Establishment of a Baltic Sea Region Blue Biotech Network
- Establishment of a “Centre for Bioprospecting Baltic Sea Microorganisms”
- Distribution networks for cosmetics/health care/wellness products from the BSR
- Development of new drugs from the BSR
- Marine genomics as source of novel enzymes from the BSR
- Novel feed from the BSR for marine aquaculture
- Innovative marine technologies from the BSR
- Innovative anti-fouling products from the BSR

Blue Biotechnology provides great potential for the Baltic Sea Region. However, a close cooperation between academia, investment and commercial organisations as well as policy is essential to successfully transfer the findings from ecological investigation to biotechnological application and marketing.

The ultimate hope of our activities in the framework of SUBMARINER is to strengthen the Baltic Sea Region’s competitiveness in Blue Biotechnology and to contribute to a sustainable development of the Baltic Sea Region.

**Figure 1:** Biodiversity of microorganisms (bacteria, fungi, microalgae) and macroorganisms (e.g. sponges, mussels, macroalgae) from the Baltic Sea as source of high-value products providing benefits for science & industry, for human health & environment as well as for growth & economy of the Baltic Sea Region.



### References

- 1 Imhoff, JF, Labes A, Wiese J. Bio-mining the microbial treasures of the ocean: New natural products. *Biotechnology Advances* 29:468–482 (2011).
- 2 Internal Co-ordination Group for Biotechnology (ICGB). OECD Working Party on Biotechnology. “Biotechnology Update” (2011).
- 3 Marine Board-ESF Position Paper 15 “Marine Biotechnology: A new Vision and Strategy” (2010).
- 4 Ecorys, Deltares, Oceanic Development. Blue Growth – Scenarios and Drivers for Sustainable Growth from the Oceans, Seas and Coasts. Third Interim report. 13 March 2012. ([http://ec.europa.eu/maritimeaffairs/documentation/studies/documents/blue\\_growth\\_third\\_interim\\_report\\_en.pdf](http://ec.europa.eu/maritimeaffairs/documentation/studies/documents/blue_growth_third_interim_report_en.pdf))

## Six priorities proposed for marine biotechnology in Denmark

Torger Børresen | DTU Food, Denmark

Denmark is surrounded by water and has a long tradition for marine and maritime activities, including exploitation of aquatic resources. The development of marine biotechnology for further improvement in aquaculture and other utilisation of resources from the aquatic environment is thus of high interest for new commercial operations. Many companies are already major players within areas such as enzyme production, fine chemicals and food additives, pharmaceuticals and ship paints, all with relevance and connection to the aquatic environment.

A survey initiated by the Ministry of Food, Agriculture and Fisheries and published in 2010 resulted in six priorities for the development of marine biotechnology in Denmark. Business opportunities were suggested and some elements for a successful strategy were proposed.



Cover page of the Danish government's report on possibilities of Blue Biotechnology and the use of marine resources

### Increased exploitation of marine biomass

In addition to traditional fisheries, full utilisation of all catches and improved utilisation of by-products are suggested. Further, new species of fish and macro algae should be harvested. The further development of this area should be seen in the perspectives of the biobased society, where all components in a given raw material are being utilised. The Strategic Research Council has suggested an increased effort to develop new energy resources through biorefineries in order to release the sources of bioenergy. Both micro and macro algae are potential raw materials for pursuing the new goals.

### New farming operations

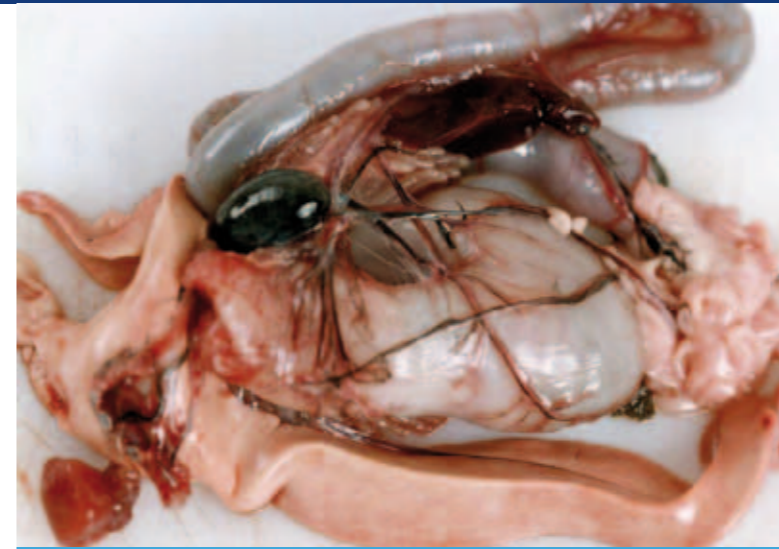
Aquaculture should be applied in its widest sense. Marine farming has the advantage that it does not impact freshwater resources. If established on land, water should be re-circulated and production limited to high priced species. Finfish species not farmed yet should be brought into production if obtaining satisfying prices on the market. For all stages of life cycle development and for controlling diseases principles of molecular aquaculture should be applied, implementing use of omics technologies and other techniques of molecular biology. Optimal development of farming the oceans is seen by introducing Integrated Multi Trophic Aquaculture (IMTA), in which species at different trophic levels interact with each other in order to minimise the effects on the surrounding environment.

### Healthy diet

Marine fatty acids, proteins, peptides and micronutrients are found in large quantities in marine organisms and should be contained directly or indirectly in the human diet. It is well established that omega-3 fatty acids are valuable nutritional components necessary for preventing heart diseases and for development of the brain, but the same fatty acids are also believed to have a number of other health effects like preventing osteoporosis and improving joint functions and central metabolic pathways. Further many new effects have been discovered for marine proteins and peptides resulting from hydrolysis of fractions considered as waste. Among the effects are antioxidant and anti cancer effects are seen such as angiotensin converting enzyme (ACE) inhibition. Some of the effects are thought to be potentiated if the lipids, proteins or peptides are acting together with non-protein nitrogenous compounds, such as taurine, occurring e.g. in high amounts in the fish gut fraction.

### Discovery of new compounds, materials and biological activities

The large variation of marine organisms and the occurrence under extreme living conditions increase the chances for new commercial viable discoveries. Many studies within marine biology are being characterised as biodiscoveries, showing potential ap-



*Denmark is surrounded by water and has a long tradition for marine and maritime activities. The development of marine biotechnology is thus of high interest for new commercial operations.*

plications of new biological principles through bioprospecting, in which commercial potentials can be developed. Many marine environments have shown habitats for life forms under extreme conditions of high or low temperature, high pressure and composition of minerals and nutrients being far from satisfying for terrestrial organisms. Many new biological principles, enzymes and components have already been discovered, but it is believed that we have seen only the beginning of this development until now. The Danish oceanic expedition around the world, Galathea 3, sampled water and sediments on an eight months cruise covering both arctic and Antarctic waters in 2006–2007.

### Extraction of valuable biochemical components

Complex structures with special properties can be included in pharmaceutical products, cosmetics and special foods. The invertebrates of the oceans are a rich source of new components and in many cases can lead us to a better understanding of life forms in organisms like e.g. sponges. The sea urchin group contains such a large variation of species that some can be used for foods while others are toxic, and others still, contain complex components that already have been used for medical purposes. As these components are studied in further details new applications within the pharmaceutical area are considered, and exam-

ples have been found of anticancer effects and components being used for treatment of e.g. malaria. In many cases the structures are so complex they cannot be made by chemical synthesis, and it may be difficult to transfer genes for production in other organisms.

### Biofilm – from ships over the food industry to the interior of the human body

Biofilm is a deposit and growth of organisms on surfaces, usually with consequences unwanted for economical or health reasons. The mechanism for building biofilms on surfaces are quite well known, but possibilities for preventing formation of biofilms are largely unknown. Obvious areas where such prevention is wanted are e.g. ship paints and surfaces in the industry, where cleaning of surfaces in the food processing industry presents many problems. A new, more controversial area is bacterial film formation inside human blood vessels observed in certain diseases.

## Trends of biotechnology in Latvia

Juris Vanags | Latvian Biotechnology Association, Latvia

The Latvian Biotechnology association (LBA) was created as the first Latvian biotech network in 2006.

The main aim of the LBA is to promote the development of biotechnology in Latvia by clustering of academia, governmental bodies and industry in joint projects, by participation in the international projects, by joint representation of their members in meetings and exhibitions, by promoting of education of young specialist in biotechnology and related fields, and by rising of public awareness on biotechnology in the society. LBA is a member of European Federation of Biotechnology and ScanBalt network.

The activities of Latvian Biotech SME, which use biotechnological methods in manufacturing and service, are oriented to the following directions:

1. The medical and pharmaceutical biotechnology;
2. The ecological biotechnological;
3. The design and manufacturing of the biotechnological equipments;
4. The Industrial biotechnology.

The biggest turnover is in the direction of the biotechnological equipments (different laboratory equipment, as well laboratory and pilot scale bioreactors), although the amount of companies

are relatively small, they have the international recognition. Now research institutions and companies consider the opportunities to start activities in directions of blue biotechnology. As the first steps is the adaptation of methods used by potential partners of EU countries.

### Potential of Latvian biotechnology

The potential of the Latvian biotechnology is in the availability of the corresponding specialists and students. Regardless of the fact, that the conditions for the more rapid development of the biotechnology are being created insufficiently intensive, which is mainly connected with the poor availability of the investments, because the local investors are oriented towards the more rapid profit bringing branches, however the foreign investors have taken the wait-and-see position.

In order to promote the more rapid development of the Latvian biotechnology, the scientific researches are to be focused in the most topical directions, it is necessary to promote the SME activities, as well as the international collaboration has to be developed in the sphere of the initiation of the larger projects, as well as it is necessary to solve the issues, connected with the investment attraction.

*The potential of the Latvian biotechnology lies in the availability of the corresponding specialists and students.*



## MAREX: Exploring marine resources for bioactive compounds – From discovery to sustainable production and industrial applications

Päivi Tammela, Paula Kiuru, Tiina Lipiäinen, Jari Yli-Kauhaluoma & Heikki Vuorela | Faculty of Pharmacy, University of Helsinki, Finland

Through close co-operation within the EU FP7 project MAREX 19 industrial and academic partners from 13 countries are exploring marine resources for novel bioactive compounds by collecting, isolating and classifying marine organisms from the Atlantic, Pacific and Indian Oceans as well as from the Mediterranean, Baltic and Arabian Seas. The main objectives of MAREX are to isolate, characterise, and sustainably exploit new compounds from extracts prepared from marine organisms that have been harvested from the seas and oceans, culture collections, or acquired from institutional, local, national, and commercially available collections. The extracts are subjected to a highly representative and diverse panel of multidimensional bioactivity assays and most promising extracts showing bioactivities will be subjected to chromatographic separation. Isolated marine compounds are characterized thoroughly in terms of analytical purity and chemical structure. The most interesting hits will be produced via sustainable biotechnological processes or structurally optimized in a sustainable medicinal chemistry programme with the aim of developing therapeutic agents to the stage where they are available for thorough early-ADME and toxicological evaluation. Furthermore, the extracts prepared from marine organisms as well as isolated fractions and pure compounds will be screened and tested for other industrially interesting applications, such as nutraceuticals, cosmetics, agrochemicals and other speciality chemicals (e.g. antibiofilm agents, antifoulants). One of the objectives is also to develop biosensors for monitoring the quality of marine organism-based food and the biotechnological production of targeted bioactive compound for process control purposes.

During the first 18 months of the project, a vast number of marine organisms, such as micro- and macroalgae, dinoflagellates, sponges, sea anemones, sea cucumbers and cyanobacteria from European, Asian and South American marine and brackish ecosystems have been collected and extracted. The crude extracts of marine organisms have been processed to isolate and chemically characterize their most potent bioactive compounds by chromatographic separation and structure identification. The main results are (i) isolation and characterization of cytostatic and apoptotic polyether triterpenoids from red seaweed *Laurencia viridis*, (ii) discovery of serine protease and protein phosphatase inhibiting oligopeptides from the Baltic cyanobacterium *Nodularia spumigena*, and (iii) discovery of theonellasterol as a potent and selective bile acid receptor antagonist.

Extracts, fractions and isolated or synthesized compounds produced during the project are being comprehensively studied for their potential use in pharmaceutical, cosmetic, food/nutraceutical, agricultural and other applications in more than 50 different biological activity assays. The key discoveries are: (i) several antiproliferative and pro-apoptotic hits have been identified, (ii) many samples have been identified as inhibitors or antagonists of thrombin, trypsin, fibrinogen receptor or vitronectin receptor, (iii) extracts with anti-inflammatory effects have been identified, (iv) synthetic derivatives interacting with sodium channels have been discovered, (v) antifungal and antibacterial screening have revealed many active samples against e.g. *Candida albicans*, *Staphylococcus aureus* and *Cryptococcus neoformans*, (vi) protein phosphatase assays have shown stimulating effects by marine organism -derived compounds.

### The MAREX consortium:

- University of Helsinki – Finland (Coordinator)
- University of Ljubljana – Slovenia
- University of La Laguna – Spain
- University of Gdansk – Poland
- Katholieke Universiteit Leuven, Belgium
- Åbo Akademi University – Finland
- American University of Beirut – Lebanon
- Universidad de Antofagasta – Chile
- University of Strasbourg – France
- Ege University – Turkey
- Università Degli Studi di Napoli ‘Federico II’ – Italy
- Universidad Catolica del Norte – Chile
- Technical Research Centre of Finland (VTT) – Finland
- National Institute of Oceanography – India
- Acreo AB – Sweden
- Xention – United Kingdom
- BioVivo – Poland
- Ebiotec S.A. – Spain
- BiotechMarine – France





Photos by Christian D. Müller (UDS) and Saliou N'Gom (BIOTECHW)



**Finland is coordinating the large-scale FP7 project MAREX – Exploring Marine Resources for Bioactive Compounds.**

The MAREX project has also studied biotechnological production of marine organisms and biosensing. The highlights of these studies are: (i) a successful optimization of the dinoflagellate *Prorocentrum lima* cultivation, (ii) evaluation of several bioreactor types for production of microalgae biomass; (iii) establishment of cryopreservation strategies for microalgal species; (iv) promising results from an aptamer selection against marine toxin okadaic acid; and (v) the development of a biomolecular detection assay and digital microfluidics.

In addition, design and synthesis of derivatives and analogs of marine bioactive compounds, their structure-activity relationships analyses, and development of the most promising hits to lead compounds have been carried out. The major results include (i) the first total syntheses of marine sulfated sterols solomonsterols A and B, (ii) compound libraries of marine compound-based derivatives have been designed and synthesized for bioactivity assays, and (iii) homology models of human voltage-gated sodium channels 1.3 and 1.4 have been built.

Research carried out so far within the MAREX project has demonstrated the potential of marine environment for offering lead compounds for pharmaceutical, nutraceutical, cosmetic, agro-

chemical, food processing, material and biosensor applications. Biodiversity in the seas is far from being fully explored. Classification of marine organisms exceeds 230,000 species; in the European seas over 36,000 species are known and 5,000–20,000 species are estimated to be still undiscovered. The maritime territory of the European Union is the world's largest with an economic zone covering 25 million km<sup>2</sup>. The Atlantic Ocean, the Mediterranean Sea, the Aegean Sea, the Tyrrhenian Sea, the Adriatic Sea, and the brackish Baltic Sea can be regarded as the most important ones for the EU. Despite the values of marine organisms, many species have become threatened by human activities. Therefore, some of the core activities in MAREX, such as developing sustainable industrial processes based on biotechnological production and synthetic chemistry, are highly important for environmentally conscious exploration of the marine environment for human benefit.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007–2013] under grant agreement n° 245137.

[www.marex.fi](http://www.marex.fi)

## Blue Biotechnology for sustainable innovations – a Blue Biotech project in the Kattegat and Skagerrak

The marine environment has great potential for providing us with food, industrial raw materials and energy, but this potential remains largely unused.

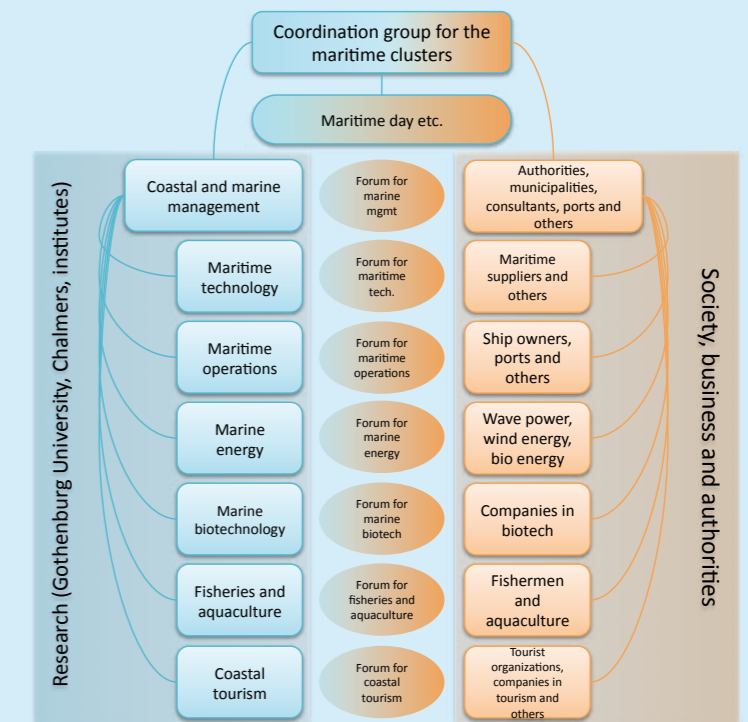
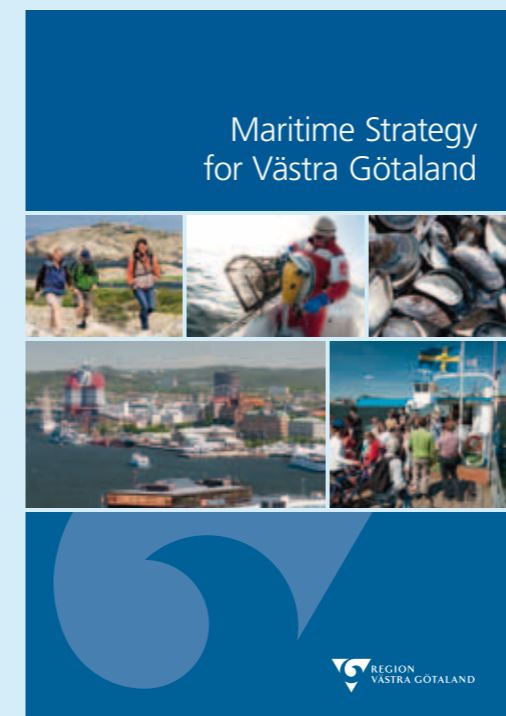
In the light of this situation and taking into account today's climate change problems in the marine environment, especially the decline of phytoplankton over the past century, the University of Gothenburg in Sweden (lead partner) has in collaboration with (among others) Mare Life in Norway developed a cross-border project in order to establish a strong research and innovation area for blue (marine) biotechnology in the Kattegat-Skagerrak region. The project, called 'Blue biotechnology for sustainable innovations', focuses on commercialization of research, enterprise integration and micro algae.

"Blue Biotechnology for sustainable innovations" is a project part-financed by the Interreg IVA-program (Kattegat-Skagerrak sub-program) of the European Union. The project runs over a three-year period, from January 2011 to December 2013.

The vision of the project is that the region around Skagerrak and Kattegat in ten years will be one of the most dynamic research and innovation locations for blue biotechnology. The region will have attracted internationally renowned scientists and the national and international research funding will have increased by a factor of 20. Furthermore, open innovation and other characteristics of the chosen strategy will have led to a prosperous industrial centre for blue biotechnology.

To help to achieve this, the project will for example establish strong academic-industrial networks and collaborations; create an attractive research environment in the 'KASK region'; enhance the knowledge of the climate effects on phytoplankton and try to find new sustainable ways of exploiting the resources that the oceans offer.- A created network between schools, companies, competence centers and universities.

[www.bluebio.org](http://www.bluebio.org)



**The Maritime Strategy for the Swedish region Västra Götaland (2008) stresses the need for more expanded research in the field of marine biotechnology in order to meet its objective of preserving marine biodiversity and the marine ecosystems' status. In 2011, a regional maritime cluster initiative was launched, involving a wide range of ecosystem goods and services from shipping to marine biotechnology (right).**

## Blue Biotechnology in Germany – the Kiel Centre for Marine Natural Products KiWiZ

Johannes F. Imhoff | GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

The local study “Sea our Future” in the state Schleswig-Holstein in 2004 and the ESF Marine Board Position Paper No. 15 on “Marine Biotechnology: A New Vision and Strategy” in 2010 both highlight the extraordinary importance of development of Blue Biotechnology in the local and the European context.

In contrast to a number of other European countries Germany currently lacks national efforts on a marine biotechnology initiative. In Germany research and development activities in marine biotechnology are scattered and studies on marine natural products lack a powerful institution to promote this topic. On the regional level, blue biotechnology has been recognised as an important field in Schleswig-Holstein and is part of activities formulated in a strategic “Masterplan Marine Biotechnology Schleswig-Holstein”. Research on marine biotechnology and/or marine natural products is a major and strategic part of just a few German institutions:

The **Fraunhofer Research Institution for Marine Biotechnology** in Lübeck has put its focus on isolation and utilisation of stem cells of fish and on integrative aquaculture, but does not include other topics of marine biotechnology and does not work on marine natural products.

The **Institute for Marine Resources GmbH imare** in Bremerhaven has its focus on biosensor technology, technical applications of marine structures and nanomaterials, but does not perform marine natural products research. It was established in 2009 and is supported by finances through EFRE and from the state Bremen.

The **Institute of Marine Resources e.V. IMaB** in Greifswald exists since 1996 and operates with participation of members of the university Greifswald. It is build as a virtual institution organised in projects. Scientific activities include functional genomics, expression systems, natural products and marine enzymes.

The **Kiel Centre for Marine Natural Product Research KiWiZ** is embedded in the Helmholtz Centre for Ocean Research GEOMAR and represents a minor activity of this centre. It was established in 2005 and supported through EFRE and the State Schleswig-Holstein finances until 2011 and is now an exclusive activity of the Research Unit Marine Microbiology of GEOMAR. It is specifically focused on marine natural products from marine bacteria and fungi and represents a major research facility of marine biotechnology in Northern Germany.

### KiWiZ’ mission and aims

The Kiel Centre for Marine Natural Products KiWiZ is a platform for natural product research from marine microorganisms covering aspects from the habitat to the hit for drug candidates and for other uses. KiWiZ aims to include research on ecological aspects of natural products as well as the sustainable biotechnological production of compounds valuable for various applications such as human and veterinary medicine, cosmetic, food or feed. It essentially works on the biology and chemistry of marine natural products.

The focus of the KiWiZ at GEOMAR is on identification, production and promotion of new natural products from marine sources and the investigation of their biological activities and ecological function. The KiWiZ was founded as an initiative of the state Schleswig-Holstein in northern Germany with the aim to establish a research platform for exploration of marine microbial resources and to search for natural products and study their biotechnical production for various applications.

The KiWiZ is incorporated into the Helmholtz Centre for Ocean Research GEOMAR (formerly the Leibniz-Institute of Marine Sciences) in Kiel.

### Research Strategy of KiWiZ

With its unique setup and resources, the KiWiZ has developed into an excellent scientific centre on new natural products from marine microorganisms. KiWiZ is operated by an interdisciplinary team of microbiologists, chemists, geneticists, pharmacists and biotechnologists. Basic and applied research represents the full value chain from the marine habitat to the biotechnology product. It is a research platform for continuous supply of new natural products from marine resources in early drug discovery. With its expertise in marine drug discovery KiWiZ represents a significant aspect of the blue biotechnology in Schleswig-Holstein, Germany and Europe.

The research of KiWiZ relies on the very high diversity of marine microorganisms either newly cultured with special intention or contained in the large culture collections of marine bacteria and fungi strains covering more than 15,000 isolates, with a high proportion of new and unknown taxa. Important aspects became the growing panel of biological assay systems, in which suitability for specific applications is tested and the establishment of a substance library of pure marine natural products. Major results are patented with priority and then published in order to safe intellectual property rights for possible industrial application. Studies of the KiWiZ include all aspects from sampling, isolation and identification of the microorganisms, their preservation in culture collections, the extraction, purification, structure elucidation and characterization of natural products from the cultured bacteria and fungi, optimisation of production conditions and scale up to a pilot scale for biotechnological production of bioactive natural products (Fig. 1).

### Research Topics of KiWiZ

Research on bioactive compounds has two major aspects. The first relates to the multiple biological functions bioactive compounds can fulfil. They may play a role in cellular communication, signalling and in the defence of predators and pathogens and even may contribute in shaping the structure of marine microbial communities.

The scientific work of KiWiZ aims to enlarge our knowledge on **the biology of natural products** by investigation of

- the diversity and potential of new marine natural products and their producers
- the role of bioactive compounds in marine microbial interactions
- the genetics and regulation of biosynthesis of marine bioactive compounds

The second major aspect related to marine natural products deals with the **potential use of bioactive compounds** in pharmaceutical applications, for crop protection, cosmetics and as food additives. This is also the most important aspect of research in KiWiZ. Marine biological resources provided by the tremendous biological diversity of marine organisms offer a great potential for human uses, in particular bioactive compounds produced by the mostly untapped microbial resources (Fig. 2).

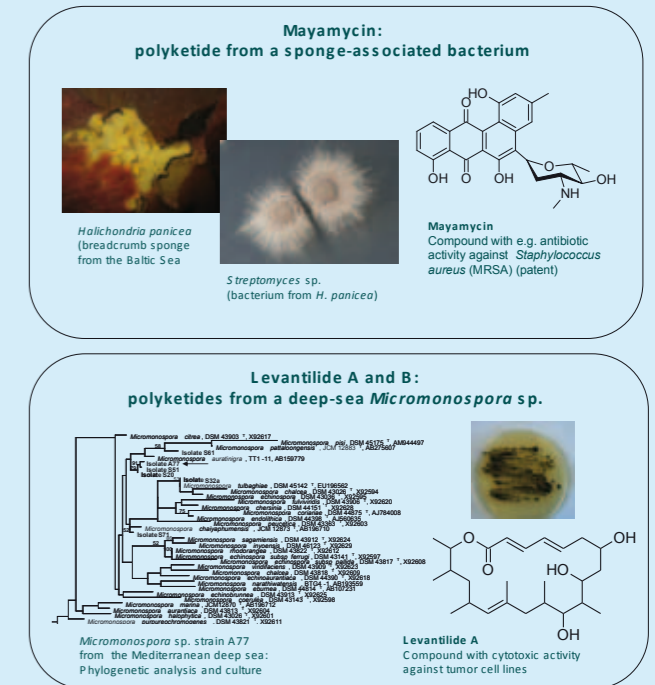


Figure 2: Examples of new bioactive compounds from the pure compound substance library of the KiWiZ (Schneemann et al., 2010. J. Nat. Prod. 73:1309–1312; Gärtner et al., 2011. Mar. Drugs 9:98–108)

The exploration of marine microbial resources, the development of methods for their biotechnological production with minimised risks for nature and man as well as their sustainable use is included in our research strategy by:

- the analysis of the genetics of marine bioactive compound biosynthesis and their application to improve substance spectra produced and production rates
- the analysis and evaluation of the biological activities of natural products as well as the development of new bioassay systems
- the development of biotechnological processes for the production of bioactive compounds

The performance of these research topics necessitates expertise in bacterial and fungal systematics, in natural product chemistry and structure analysis, in bacterial and fungal genetics and physiology, in pharmaceutical targets, in fermentation technology and process development. In addition basic, support is required to maintain microbial culture collections and chemical substance library.

### KiWiZ as a Thriving Force for Networking and Promotion of Marine Biotechnology

The KiWiZ is engaged in partnerships with national and international academic research facilities and commercial enterprises to promote natural products to the market for pharmacy, cosmetics, plant protection and food development. The KiWiZ offers the broad spectrum of available methods including bioassays, chemical structure analysis, identification of microorganisms and fermentation to academia and industry in the framework of cooperation projects.

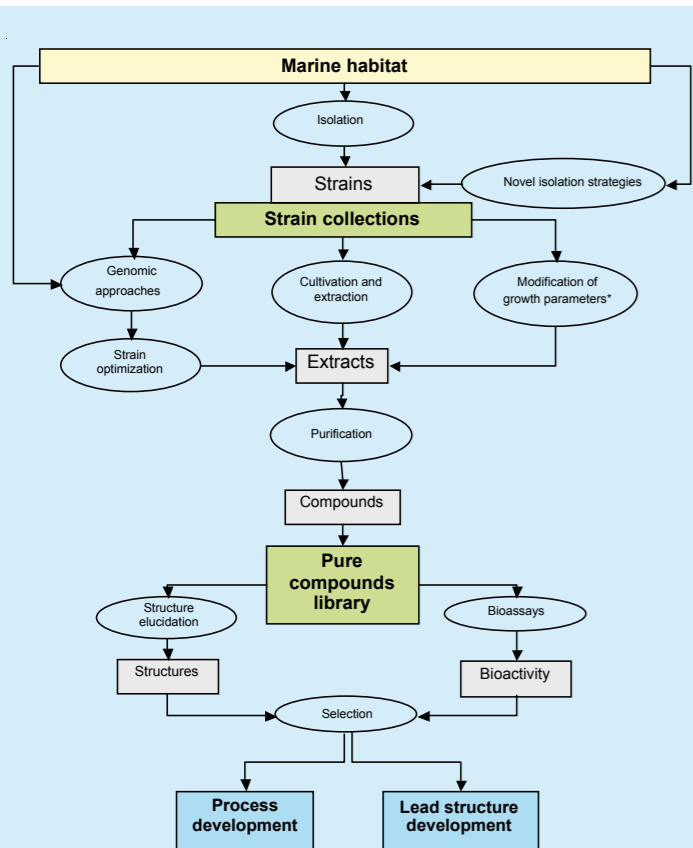


Figure 1: Strategic outline of the research profile of KiWiZ for a sustainable discovery and development of marine microbial natural products (Imhoff et al., 2011, Biotech. Adv. 29:468–482).

## Spila Spirulina microalgae applications for human food, health and animal feed

Vytas Rimkus | SPILA, UAB, Lithuania

Most people know that algae grows in oceans, seas and also lakes. There are more than 30,000 species of algae which have been around for billions of years. Algae represent two thirds of the earth's biomass. Spirulina is a blue-green microalgae naturally thriving in alkaline lakes. It is the most concentrated and nutritious whole food known to science.

Spirulina has no nucleus and its cell walls are soft and easily digestible, unlike those of other plants that contain hard cellulose, making it difficult to digest and utilise all their nutritional values. A rich nutritional content and a good balance of all nutrients make it a perfect source for human food, health as well as animal nutrition.

### Benefits of using Spirulina

Several hundred scientific studies and clinical trials indicate health benefits associated with the use of Spirulina. Spirulina is the perfect multinutrient food for people of all ages and lifestyles who want a whole food source of vitamins and other nutrients.

There are many benefits of using Spirulina:

- Spirulina thrives in alkaline lakes where it is difficult or impossible for other microorganisms to survive and can be cultivated on poor or deserted lands not suitable for agriculture;
- algae can double its biomass every 2 to 5 days;
- productivity breakthrough yields over 20 times more protein than soybeans on the same land area, 40 times more than corn and 400 times more than beef;
- Spirulina protein uses 1/3 the water as soy, 1/5 as corn, and only 1/50 the water needed for beef protein.

Spirulina is not only good for people, but animals and plants thrive on it, too. Many scientific trials – including some conducted by our company together with partner scientific institutions – indicate that Spirulina can be economically efficient and viable in animal nutrition and health applications.

When given Spirulina, old cats and dogs with dull, thinning coats have been seen to develop thick, lustrous coats; and pets with stiff joints appear to improve considerably, becoming supple and active again.

Veterinarians prescribe Spirulina to aid recovery, increase stamina, relieve stiffness, and also for show animals for general toning, as these animals need to look their best.

Prize winning koi carp are fed Spirulina to enhance their color and health. Bird breeders add Spirulina to feed to enhance plumage color and luster.

Spirulina is used extensively by aquaculture companies to improve the growth rates, increase disease resistance, improve survival rates, reduce medication requirements and improve quality and coloration of various fish and shellfish.

### Feed additive for dairy cows

SPILA, UAB applied scientific work carried out by a number of international scientists in the field of human nutrition and animal productivity and economical efficiency. Areas of trials were Spirulina for dairy cows, use for poultry, pig, carp fish and bees.

Hence we created SPILAMIX, a feed additive for dairy cows. SPILAMIX is a unique feed additive for dairy cows with a special component Spirulina that improves the functions of the animal's organism and stimulates its productivity.

### The minimum and maximum economic SPILAMIX advantage in the dairy cows' holdings (EUR), after product's cost deduction

Minimum advantage (milk procurement price 0.2 EUR/l, yield increase 3.9l/cow)					
Number of cows	10	50	100	200	500
1 month	114	570	1,140	2,280	5,700
2 months	228	1,140	2,280	4,560	11,400
3 months	342	1,710	3,420	6,840	17,100
6 months	692	3,458	8,372	17,472	34,350
12 months	1,387	6,935	13,870	27,740	69,350

Maximum advantage (milk procurement price 0.35 EUR/l, yield increase 6.5l/cow)					
Number of cows	10	50	100	200	500
1 month	563	2,813	5,625	11,250	28,125
2 months	1,125	5,625	11,250	22,500	56,250
3 months	1,688	8,438	16,875	33,750	84,375
6 months	3,413	17,063	34,125	68,250	170,625
12 months	6,844	34,219	68,438	136,875	342,188

The Lithuanian company SPILA uses microalgae applications for human food, health and animal feed products.



SPILAMIX has many features and benefits:

- promotes milk yield increases (up to 6.5l of milk/cow/day);
- has a positive effect on milk quality, reduces the number of somatic cells up to 26%; improves the animal's digestive and metabolic processes (up to 5.2%) and helps assimilate some trace elements;
- strengthens resistance to diseases (e.g. mastitis);
- increases the quantity of erythrocytes (up to 19.3%  $10^{12}/l$ ), hemoglobin (up to 20.3% g/l) and hematocrit (62.4%) in the blood, thus reducing the number of leucocytes (up to 39.3%);
- is a high quality product that is free of harmful chemicals and side effects.

The use of unprocessed food rich in natural vitamins has become a trend, as its importance in response to obesity and different widespread lifestyle diseases (cardiovascular for instance) has been understood.

Understanding this, we created a natural preservation and digestion enhancement Spirulina based product on maximum nutrient preservation and without thermal preservation thus

suitable for vegetarians and everyone from children to elderly senior. As it represented unique combinations which were not used before it was internationally patented. It proved to be successful for its health benefits as well as for its taste which made it a part of functional food.

For animal nutrition, our task was to increase benefits for the use of natural Spirulina additive versus synthetic or chemical animal productivity enhancer. We achieved a significant increase in milking cows' milk yield and other benefits resulting in 50%–400% net return per day investment on our new SPILAMIX product. This is hard to match analogue in this industry.

### Outlook

As Spirulina represents hundreds of different forms of nutrients and a whole system of interaction, it is a great object for further studies for exploring its further benefits for human and animal and to enhance its further utilization in many applications also as a source of vital elements.

International co-operation in patent sharing and further product development is a main business objective for our company.

## Blue Biotechnology Research Centres in Poland

Alicja Kosakowska & Adam Żak | Institute of Oceanology, Polish Academy of Sciences, Poland

Blue Biotechnology is a dynamically developing branch of science. Application of molecular biology methods to marine and fresh-water organisms is a worldwide trend which could lead to new important discoveries. This modern approach brings many opportunities along with possible practical applications and benefits for human medicine, pharmacy and industry. The main goal of this article is to introduce and describe some of the scientific institutions in Poland that are involved in blue biotechnology – taking into account both research projects and future perspectives.

### Screening for biologically active compounds in marine environment

The work at **Laboratory of Biochemical Ecology of Microorganisms of the University of Gdańsk's Institute of Oceanography** is focused at three strictly interwoven activities:

- isolation, cultivation and biomass production of cyanobacteria and microalgae,
- screening of extracts for biological activity,
- and activity guided fractionation, isolation and identification of bioactive compounds.

The biological material for the studies is isolated from marine, brackish and freshwater environments. In the culture collection, more than 150 microorganisms are maintained under conditions optimal for growth and metabolites production. Biological activity of crude extracts, fractions and isolated compounds is analyzed using an array of biological and biochemical assays. The aim of the work is to estimate the potential application of natural compounds as pharmaceuticals e.g. of antimicrobial, anticoagulant and cytotoxic activities. Additionally, growth regulating activity (herbicidal and algicidal) of the compounds is tested. Characterization of chemical structure of natural products is based on LC-MS/MS analysis. Mass spectrometry is also used to determine metabolic diversity within cyanobacterial genera and species. At the moment, the work on marine biotechnology in the laboratory is conducted within one European project (FP7 project MAREX, see p. 13–14) and one national project.

### Detection of mutagenic pollution of marine environment by using genetically modified marine bacteria

Mutagenic pollution of the environment is a global and important problem which also occurs in the marine environment. Although many mutagenicity assays have been developed, there are specific problems with testing marine water and sediments

for mutagenic contamination. One of them is the fact that most genetically modified strains used in commonly available microbiological mutagenicity assays, like *Escherichia coli* or *Salmonella*, survive relatively poorly in marine waters, especially those of higher salinity. At the **Department of Molecular Biology** of the **University of Gdańsk**, two alternative assays have been developed, in which bacteria occurring naturally in marine habitats are employed.

The first assay is similar to the Ames test. However, a set of genetically modified, neomycin-sensitive *Vibrio harveyi* strains is used. Mutants resistant to this antibiotic can be easily isolated and the frequency of the appearance of such mutants increases in the presence of mutagens. To enhance sensitivity of the assay, a transposon mutant, very sensitive to mutagenic factors, was isolated. The second modification was the introduction of a plasmid bearing *muca* and *mucB* genes, coding for proteins involved in an error-prone DNA repair. The assay consists of detection of neomycin-resistant mutants on plates either containing a mutagen in the solid medium or after incubation of bacterial cultures in a liquid medium, in the presence of tested compounds or environmental samples. Neomycin is an aminoglycoside antibiotic that interferes with decoding at the ribosomal A site during translation. Resistance to this antibiotic occurs as a result of various rRNA modifications in the decoding site. Therefore, a large spectrum of mutagenic agents, causing different types of mutations, may lead to the appearance of neomycin-resistant mutants, which can be detected.

The second assay is based on the use of the *V. harveyi* mutant in the *luxE* gene. This mutant is dim, but upon contact with mutagens, fully luminescent revertants or pseudorevertants appear, thus luminescence of a bacterial culture became significantly increased. It was demonstrated that this increase in luminescence is effective and easily measurable after just a few (2–4) hours of treatment with various mutagenic agents, revealing a dose-response correlation. Although this assay resembles previously described and commercialized Mutatox, its advantage is a short time of the analysis (a few hours in this assay, relative to 24 hours in Mutatox). Usefulness of the *V. harveyi* luminescence mutagenicity assay in testing environmental samples has been demonstrated for different materials (marine water, sediments, marine plant and animal tissues) alone, as well as in combination with some other methods.

### Bioactive compounds from Baltic bacteria, cyanobacteria and microalgae

The **Marine Biochemistry Laboratory** is a part of the **Marine Chemistry and Biochemistry Department** at the **Institute of Oceanology of the Polish Academy of Sciences**. Strategic Directions of the institute's research are:

- role of the oceans in climate change and its effects for the European Seas,
- natural and antropogenic variability of the Baltic Sea environment,
- contemporary changes of the coastal ecosystems in the shelf seas;
- genetic and physiological mechanisms of functioning marine organisms, principles of marine biotechnology.

The research conducted in Biochemistry Laboratory concentrate on the following major topics:

- factors influencing transport of metal ions through cell membranes and distribution within cells (at present iron),
- development and maintenance of an algal culture collection including axenic cultures isolated from the Baltic Sea,
- short and long term trends in phytoplankton of the Baltic Sea; pigments of phytoplankton as ecophysiology and chemotaxonomy indicators,
- isolation and characterization of proteins and siderophore-like substances (natural iron chelators),
- allelopathic interactions between phytoplankton species and secondary metabolites with biological activity produced by cyanobacteria and microalgae.

The studies about secondary metabolites with allelopathic activity are important because of their possible influence on aquatic ecosystems and potential practical application in pharmacy, medicine, food science and other branches of industry.

Blue Biotechnology is a dynamically developing branch of science in Polish research institutions.

### More information & contacts

Laboratory of Biochemical Ecology of Microorganisms, Faculty of Oceanography and Geography, University of Gdańsk

Prof. Hanna Mazur-Marzec, head of the laboratory

[www.ocean.ug.edu.pl](http://www.ocean.ug.edu.pl)

Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdańsk & Medical University of Gdansk

Prof. Ewa Łojkowska, head of the laboratory; Dr Krzysztof Waleron

[www.biotech.ug.edu.pl](http://www.biotech.ug.edu.pl)

Department of Molecular Biology, Faculty of Biology, University of Gdańsk

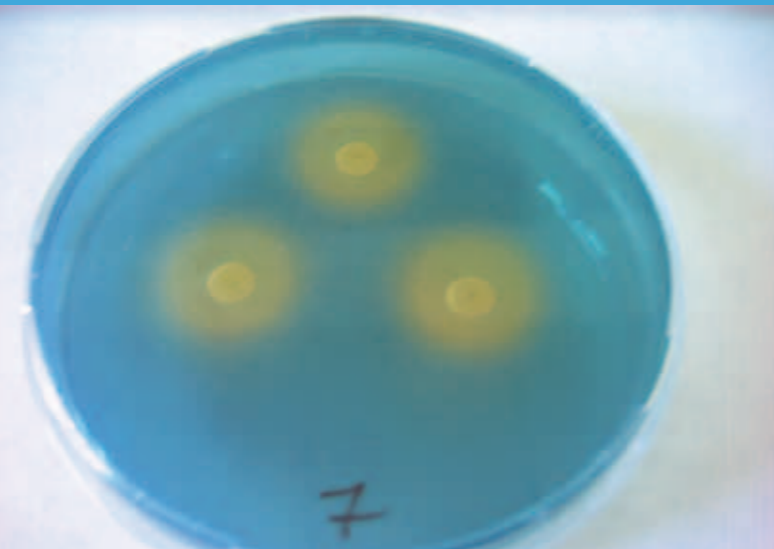
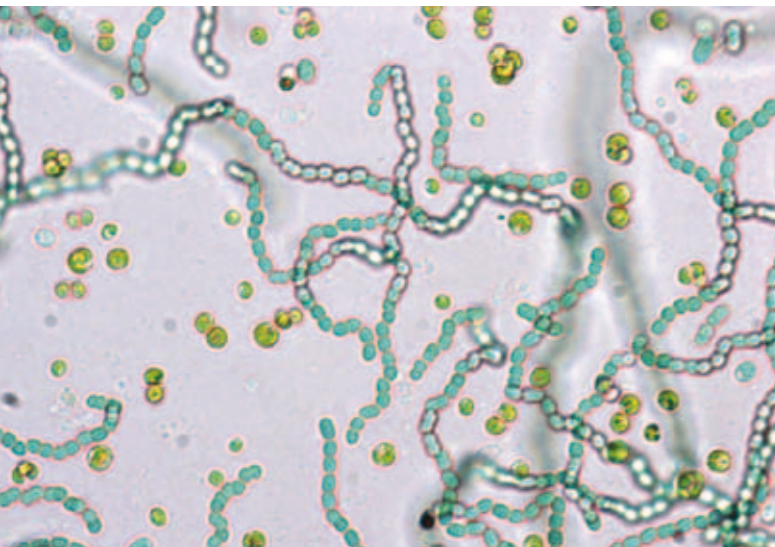
Prof. Grzegorz Węgrzyn, head of the department

[www.biology.ug.edu.pl](http://www.biology.ug.edu.pl)

Marine Biochemistry Laboratory, Marine Chemistry and Biochemistry Department, Institute of Oceanology Polish Academy of Sciences

Prof. Alicja Kosakowska, head of the department and laboratory

[www.iopan.gda.pl](http://www.iopan.gda.pl)



# Blue Biotechnology – Science meets Industry



Blue Biotechnology encompasses the applications of biotechnology tools on marine resources. With its horizontal scope, Blue Biotechnology covers very different applications, for all of which the marine environment is providing the resources. At the SUBMARINER Blue Biotechnology Cooperation Event the focus was on four different fields of applications: Genetics, Food, Cosmetics and Pharmaceuticals. Products developed in these areas have successfully entered the market during the last years. One example is the nucleosid Vidarabine (Ara-A) which was originally discovered in a marine sponge and serves as an antiviral drug for the treatment of herpes simplex infections. For the food sector, one important product is the polysaccharide alginate from a brown algae used e.g. as thickening agent. Also in the field of cosmetics, “blue” products are used. The beta-carotene produced by different microalgae function as an antioxidant counteracting premature ageing of the skin. Finally genetic tools help to investigate and understand marine organisms, which create the basis to find e.g. new enzymes or substances also from uncultivable organisms.



Given that Blue Biotechnology is a relatively young discipline with an enormous development potential, it is obvious that a lot of Research & Development work still needs to be done. The broad variety of presentations given in the “Science meets Industry” session demonstrated that this is indeed happening in all these fields of application. Different speakers from all over Europe presented topics ranging from knowledge capture mechanisms to cultivation of fish cells or successful examples within the pharmaceutical industry. Business companies, research institutions as well as regional and trans-European projects were introduced.



Presentations dealing with functional genomics, the production of omega-3 fatty acids and natural product discovery showed that the Baltic Sea Region seeks to keep pace with the rest of Europe. As a showcase of an already successfully operating commercial application from the Baltic Sea Region, a company producing organic cosmetics in Kiel shared its experience with the participants.

The “Science meets Industry” session provided a fruitful basis for active networking and discussions on prospective collaboration, which will be needed to develop the future of Blue Biotechnology in the Baltic Sea Region.

## Biotechnological advances in discovery and biosynthesis of bioactive secondary metabolites in marine microalgae

Allan D. Cembella | Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

Eukaryotic microalgae have been extensively studied for biotechnological applications but the pharmaceutical potential of bioactive compounds of marine species has scarcely been explored. This is in spite of the fact that >120 species of marine eukaryotic microalgae are known to produce bioactive metabolites. These allelochemical interactions thus provide a plausible discovery and screening approach for novel bioactive metabolites, including insights into pharmacological mode of action.

### The Chemical Ecology Strategy

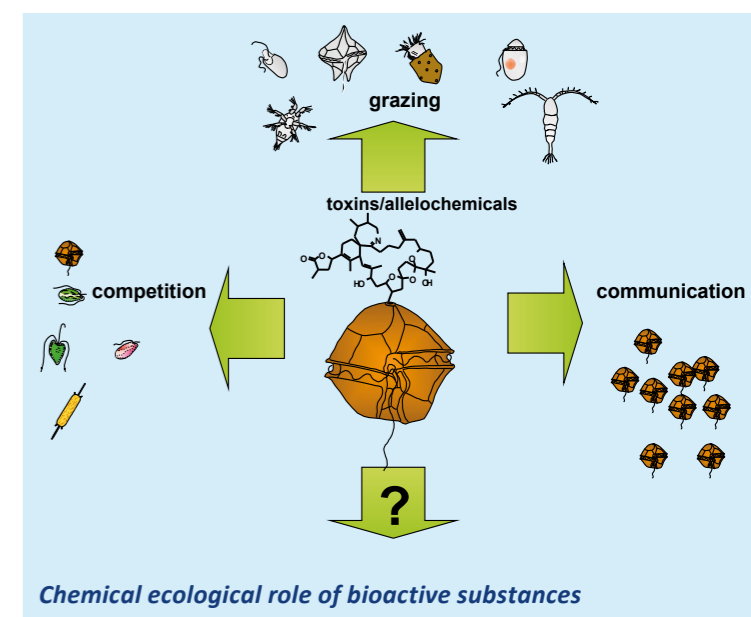
Many microalgae produce potent toxins or other bioactive allelochemical substance with detectable and often quantifiable effects on other components of marine ecosystems, such as grazers or competitors. Although the mode of action and functional role of these compounds remains poorly understood, bioeffects-based screening can be an efficient way to enhance the probability of discovery of novel bioactive metabolites from selected target species. The chemical ecology strategy begins with observations of chemically mediated species interactions in natural marine environments or in scaled-down systems (microcosms and mesocosms). This chemical ecology guided approach is a more focused alternative to blind screening for bioactivity from a bewildering array of potential candidate species.

### Culture and Harvest Opportunities

Marine microalgae are attractive candidates for biotechnological exploitation because they are usually amenable to isolation and scale-up for mass culture and can also be harvested directly from marine environments for optimal yield of bioactives. Key microalgae may form dense cellular aggregations or “blooms” within the water column or at the ocean surface, which can often cause water discoloration (*red tide*). In the case of toxic or noxious species, such blooms can lead to serious negative ecological and human health consequences. In the search for novel bioactive substances, these dense *Harmful Algal Blooms* can



Toxid red tide on the Norwegian coast



often be harvested from virtually monospecific layers by high volume pumping and filtration, e.g. through plankton nets, to yield sufficient biomass for extraction and purification of these compounds.

Most toxigenic microalgae are primarily phototrophic and therefore can be mass-cultivated successfully for biotechnological purposes in a variety of photobioreactors, ranging from simple batch-culture systems, such as glass or plastic carboys, aquaculture cylinders or suspended plastic bags, to more sophisticated continuous-flow chemostats and turbidostats with highly regulated environmental parameters and automated harvesting in continuous or semi-continuous mode.

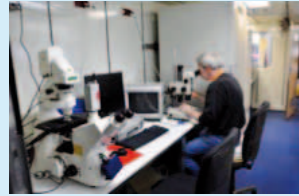
### Molecular Diversity of Bioactive Compounds

The known bioactive compounds of eukaryotic marine microalgae comprise a wide diversity of metabolite groups, from linear and polycyclic ethers (e.g., brevetoxins) to tetrahydropurine alkaloids (e.g., saxitoxin) and secondary amino acids (e.g., domoic acid). Marine flagellates, and in particular the free-living dinoflagellates, account for >80% of the known bioactive metabolites belonging the polyether class and presumably derived via polyketide pathways. With few exceptions, these compounds tend to be constitutively produced (when present) within a microalgal strain – they are not classic inducible stress metabolites – but their distribution is typically inconsistent within a genus and even among populations or strains within a species. Nevertheless, most strains produce a suite of related compounds from a given structural group, rather than a single analogue, and the composition appears to be genetically fixed, but the quantity per cell may vary widely depending upon growth stage and environmental conditions. These compounds are usually classified as “toxins” because of their high potency to higher vertebrates,

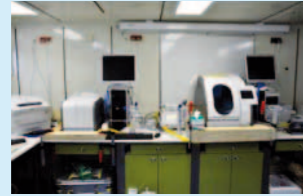
**Shipboard bioprocessing and search for marine bioactives from plankton**



Liquid chromatography – tandem mass spectrometry



Cell isolation, cloning, identification and culture



Roche 454 pyrosequencing of DNA amplicons

particularly mammals and birds, although the ecological and evolutionary role (e.g., as chemical defence agents) has not been clearly demonstrated. Particularly important from the pharmaceutical perspective is the fact that many of these toxic compounds act in mammalian systems as ion-channel effectors or as enzyme inhibitors, e.g. of protein phosphatases. Such metabolites therefore warrant further investigation regarding their potential as analgesics, tumour-promotion/inhibition agents and cell cycle regulators.

In addition to the known toxins of algal origin, known as phycotoxins, marine microalgae produce other allelochemicals with the capacity to affect species interactions by causing immobilization, loss of membrane integrity and/or cell lysis. These allelo-

chemical are structurally distinct from the phycotoxins and can comprise larger molecules or macromolecular complexes; the molecular targets are unknown but these compounds may also provide a fruitful inquiry into bioactive compounds of pharmaceutical interest.

**Ecogenomics and Functional Genomics Approaches to Biosynthesis**

The genomic revolution and post-genomic advances in understanding structural and functional gene relationships within and among microalgal taxa have already yielded profound insights into gene expression and biosynthesis of bioactive metabolites. Certain toxic metabolites (e.g. saxitoxin analogues) produced by marine dinoflagellates are shared with cyanobacteria and similar non-ribosomal peptide synthases (NRPS) and polyketide synthase (PKS) genes have been identified. The relative simplicity of the cyanobacterial genome can thus provide model systems for genomic studies of biosynthetic genes from much more complex marine dinoflagellate genomes. An effective strategy for discovery and screening for bioactives from marine microalgae integrates classical natural products chemistry (LC-MS/MS, NMR) with genomic and gene expression aspects. Taxonomic gene probes and gene expression platforms have been developed for several groups of eukaryotic microalgae and associated bioactive gene pathways. Application of high-throughput (e.g., Next-generation 454)-sequencing, DNA microarrays, and real-time quantitative polymerase chain reaction (qPCR) technology to microalgae capable of producing bioactive compounds provides an effective screening approach to discovery of novel taxa and genetic regulation of bioactive compounds in both laboratory cultures and complex field assemblages.

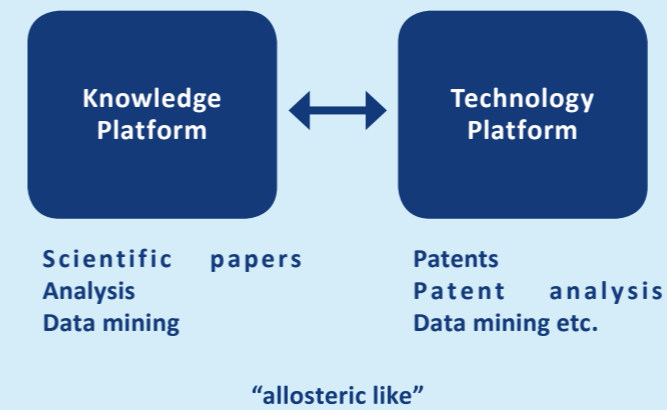
**Knowledge capture mechanisms studies as a tool to facilitate European Blue Biotech analysis**

**Daniel Pardo** | CNRS/MNHN, France  
**Sophie Arnaud-Haond** | Ifremer, France  
**Jesus M. Arrieta** | CSIC-UIB, Spain  
**Antoine Schoen** | Paris Est, France

Knowledge Capture Mechanisms (KCM) are very similar to “Allosteric reaction” involving different parameters, control and feedback control. Analysis of KCM, at the core of technology transfer, contributes to a better understanding of interactions between university and industry, technology and knowledge.

The present communication is focused on KCM involving Marine Genetic Resources and results obtained by Sophie Arnaud-Haond et al. It will present a research project and preliminary results describing some examples of knowledge transfer pathways.

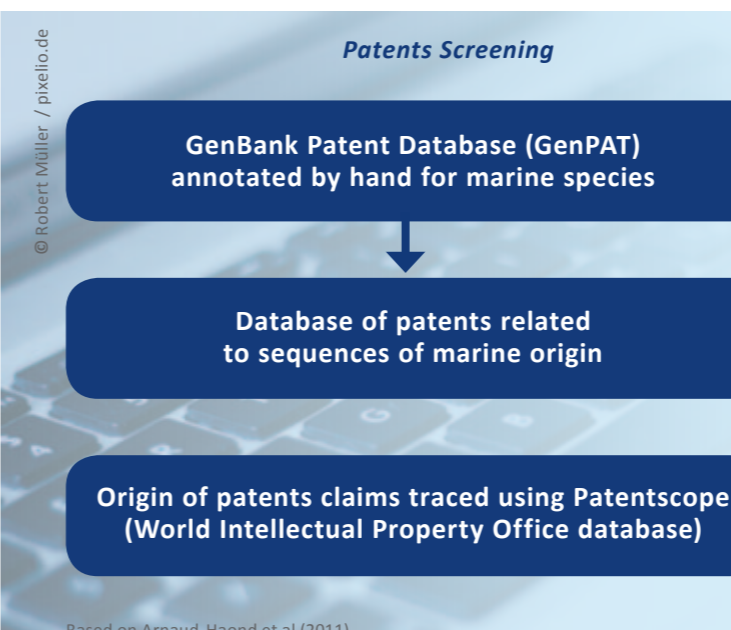
**Knowledge Capture Mechanisms**



**Patent Claims for a Gene of Marine Origin with Source**

Country	Marine organism patent claims
USA	199
Germany	149
Japan	128
France	34
United Kingdom	33
Denmark	24
Belgium	17
Netherlands	13
Switzerland	11
Norway	9

Data from Arnaud-Haond et al (2011).



Based on Arnaud-Haond et al (2011).

**BASF scientific collaborations developed by researchers mentioned as inventors in BASF marine biotech patents**



**References:**  
 Arnaud-Haond S, Arrieta JM, Duarte CM (2011) Marine Biodiversity and Gene Patents. Science 331: 1521–1522.  
 Arrieta JM, Arnaud-Haond S, Duarte CM (2010) What lies underneath: conserving the oceans’ genetic resources. Proc Natl Acad Sci U S A 107: 18318–18324.

## Marine functional genomics

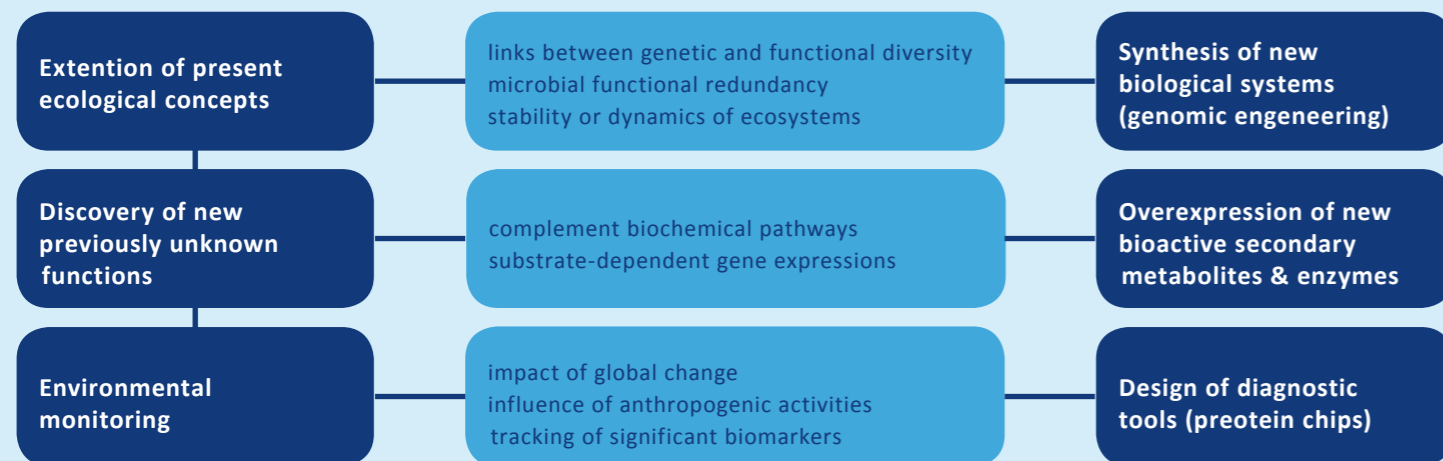
Antje Gardebrecht | Ernst-Moritz-Arndt University & Institute of Marine Biotechnology, Germany

Facing the fact that to date most microorganisms remain uncultured, deep metagenome sequencing is indispensable to get an impression of microbial diversity and physiological potential in a natural habitat. Depending on large specific genome data sets, functional approaches such as metaproteomics have been applied to characterize the dynamic range of *in situ* expressed genes. Starting with a simple microbial assemblage as the symbiosis of deep-sea tubeworms and sulfide-oxidizing bacteria, we targeted on whole proteomes to resolve functional variations between monospecific bacteria residing in two different hosts.

Challenged by rapid advances in MS/MS-based technologies, we furthermore made an attempt to investigate complex microbial communities in marine surface waters. Functional genomics al-

lowed to directly link bacteria with metabolic and biogeochemical processes in the North Sea by indication of proteomic biomarkers. Future analyses of model organisms grown with habitat-specific substrates or being exposed to different stress- and limitation factors should give a more detailed picture of key proteins in marine systems. In this context, several visualization concepts are used to compare expression patterns of such individual marker genes under defined environmental conditions. Novel identified proteins of unknown function are finally characterized and exploited in respect to new biotechnological applications.

### Biotechnological potential of functional genomics



## Fungi associated to Mediterranean seagrasses and algae: diversity, ecological role and potential exploitation

Giovanna Cristina Varese, L. Panno & G. Gnani | University of Turin, Italy  
A. Kramer & A. Labes | Kieler Wirkstoff-Zentrum am GEOMAR, Germany

Marine environments host a huge biodiversity of microorganisms and a big part of them is made up by fungi that show a high biodiversity, a specific habitat composition and seem to be very important from both an ecological and biotechnological point of view.

The basic knowledge on distribution and ecological role of marine fungi is still scarce. Marine fungi encompass a wide range of saprotrophs, parasites, symbionts, endophytes and epiphytes. They can be found in every kind of marine habitats including marine microbial communities, marine plants (i.e. algae, seagrasses, driftwood), marine invertebrates (sponges, coral, ascidians, bivalves, crustaceans, etc), vertebrates (mainly fishes) and inorganic matter<sup>1</sup>. Recently it has been estimated that marine fungi should exceed 10,000 species/phylogenies and that many poorly investigated sources of marine fungi still exist i.e. algicolous fungi<sup>2</sup>.

Moreover marine fungi are a prolific source of new chemical diversity and so far have provided many extremozymes and more than 1,000 new natural products. Many of these compounds are structurally unique and possess interesting biological and pharmacological properties. Among them polyketides play a dominant role, and if prenylated polyketides and nitrogen-containing polyketides are taken into account, their total share will exceed 50% of all new natural products from marine fungi<sup>3</sup>. However the ecological role of these natural compounds is still to be investigated. These metabolites have specific functions in the interrelations between fungi and other organisms. They are considered to play an important role in shaping the community structures and in mediating interactions among microorganisms and between microorganisms and their hosts<sup>4</sup>.

The aim of this study was to isolate and identify fungi associated with the seagrass *Posidonia oceanica* and to compare it with the ones of the green alga *Flabellia petiolata* and the brown alga *Padina pavonica* which grow in the same phytocenosis. Moreover about 200 isolated fungi have been screened for the production of enzymes and secondary metabolites of pharmaceutical interest.

Samples were collected in two sites located in the northern and southern part of the Elba island (Italy) at two different bathymetries. Plants and algae were serially washed with sterile seawater, homogenized, diluted and plated on different oligotrophic media. Plates were incubated at two different temperatures (15 °C and 25 °C) and checked daily for isolation of colonies that have been identified by morphological and molecular tools.

Despite the two algae are very different from a morphological and ecological point of view the total fungal load was very similar in all the sites. However from a qualitative point of view the two mycofloras are completely different.

As regard *Posidonia oceanica* the four districts (leaves, rhizomes, roots and fluctuating leaves) were separately analyzed and displayed great differences in load and mycoflora composition. Most of fungi were selectively associated with a district and only two species were present in all districts. Rhizomes display the highest load whereas roots and leaves the lowest one.

In accordance with literature, most of the isolated species belong to Ascomycota and represent saprotrophic species. Noteworthy from an ecological point of view the finding of several species that in terrestrial habitats usually establish pathosistic



Figure 1: *Posidonia oceanica* (left); *Flabellia petiolata* (top right); c) *Padina pavonica* (bottom right)

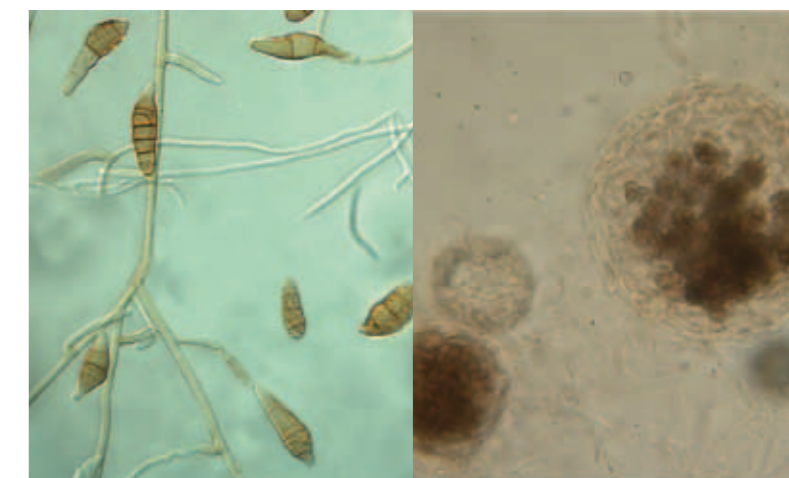


Figure 2: *Conidia of Alternaria alternata* isolated from *Posidonia oceanica* (left); *Cleistotecia of Emericellopsis minima* isolated from *Flabellia petiolata* (right).

(plant or enthomopatogenic species) or mutualistic symbiosis (lichenicolous fungi) with different organisms. Further studies will be necessary to understand their role in marine environments.

As regard the potential biotechnological applications, about 100 strains mainly isolated from *P. oceanica* were screened for the capability to produce laccases, peroxidases and tannases in absence/presence of different NaCl concentrations (15 and 30 g/l). Most of the strains were able to produce the tested enzymes; many of these enzymes are “extremozymes” since they are triggered only by high salts concentrations. These enzymes could be new isoforms very useful in various industrial applications.

Moreover 98 fungal strains isolated from the analyzed seagrass and algae were screened for the presence of two types of polyketide synthases genes (PKS) whose presence is considered predictive of the capability to produce useful pharmaceutical metabolites. The same strains were also used in an antimicrobial screening towards algae, fungi and bacteria potentially pathogen for human beings and animals.

About half of the fungal strains showed the genes involved in the PKS production. Most of fungi positive for PKS genes come from the two algae. This intriguing result may find an explana-

tion from the ecological point of view: *P. oceanica* is a seagrass very rich in tannins, molecules which display high antimicrobial activity and involved in competitive interactions with other algae (i.e. *Caulerpa taxiflora*). On the other hand, it is perhaps possible to assume that the two algae *P. pavonica* and *F. petiolata* use the fungi associated with them to defend themselves against predators and biofouling.

As regard the antimicrobial screening, several strains display remarkable antimicrobial properties towards bacterial and fungal strains while only a few strains showed antialgal activity.

#### References:

- <sup>1</sup> Jones 2011a. Fungal. Divers. 50: 73–112.
- <sup>2</sup> Jones 2011b. Bot. Mar. 54: 343–354.
- <sup>3</sup> Rateb & Eleb, 2011. nat. Prod. Rep 28. 290–344.
- <sup>4</sup> Imhoff et al., 2011. Biotechnol. Adv. 29: 468–482.

## Knowledge transfer from environmental genomic science to marine biotechnology – big data, big challenges

Johanna B. Wesnigk | EMPA Bremen, Germany

Genomic sequence data are increasing exponentially, also for marine organisms and metagenomic data sets. These include information for novel functions and promising variety of known and utilised enzymes. While costs for the sequencing are decreasing, it still constitutes a major effort to obtain and maintain samples. Therefore cooperation between science and industry is necessary.

Several EU-funded projects address how to facilitate access to data, samples and expertise. A dedicated focus on knowledge transfer to industry is provided by the CSA project “Marine Genomics for Users”, which deals with training, a knowledge output database and with establishing direct contacts between genomic scientists and marine biotechnologists. Several elements of this knowledge transfer strategy and a short overview on related projects and their outputs are given in the following.

Furthermore, the emerging large collaborative project Micro B3 “Marine Biodiversity, Bioinformatics and Biotechnology” will be presented. It concentrates on integrating genetic and ecological information in one open-access system for linking (meta)genomic prediction to ecosystem biology and to biotechnological aspects. Related training will focus on bioinformatic needs by these two user groups, including also governance and IPR aspects.

The ultimate objective is to learn from marine diversity – from fishing bio-active molecules to cultivating organisms and maybe soon synthesizing genes – to decipher and apply novel functions found in the marine environment.



The aquaculture sector is among the target groups of the “Marine Genomics for Users” project

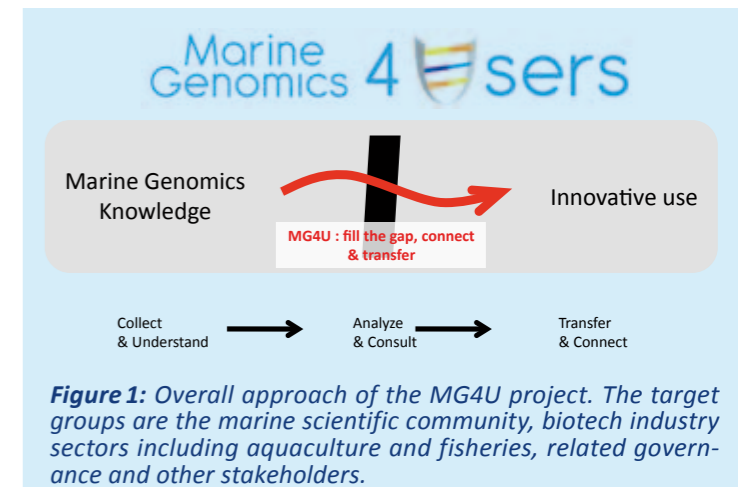


Figure 1: Overall approach of the MG4U project. The target groups are the marine scientific community, biotech industry sectors including aquaculture and fisheries, related governance and other stakeholders.

### Marine Genomics for Users

A consortium of seven partners was created to enable wise use of the Marine Genomics potential for innovative socio-economic development. Members are CNRS (Station Biologique de Roscoff), France, as coordinator; UCC (Environmental Research Institute) and AquaTT, Ireland; UGOT (Sven Lovén Center), Sweden; Dr. Johanna B. Wesnigk, EMPA Bremen, Germany; IRTA, Spain; CCMAR, Portugal.

One of the work packages (WP4) focuses especially on the transfer of marine genomics knowledge to industry. It is defining efficient methods to establish contacts and designing products for transfer of MG information to target relevant industrial sectors for (joint) development of marine genomics applications.

Means for the transfer of marine genomics knowledge are direct contacts, (e.g. via partnering), dedicated MG sessions at industry conventions, focussed industry workshops (WP3), and a MG knowledge output database (WP2). Finally uptake of knowledge and its further usage will be evaluated, in WP4 especially in the light of enhanced planning of future industry-academia collaborations.

Methodology (for more detail see [www.MG4U.eu](http://www.MG4U.eu)):

1. Short questionnaires were distributed electronically and during partnering meetings to industry contacts at several events (BioMarine, Biotechnica) to help define the needs of industry sectors, selected from white, green and red biotechnologies, fisheries and aquaculture.
2. Relevant events were chosen for promoting Marine Genomics in dedicated sessions, for example BioMarine and Biotechnica 2011, Achema June 2012 with a focus on the Bio-Based Economy and BioMarine October 24-25 2012 in London, where marine genomics will be featured in two think tanks ([www.BioMarine.org](http://www.BioMarine.org))



Figure 2: Map of geographical distribution of the 32 partners of Micro B3 large collaborative project

**Marine Biodiversity, Bioinformatics and Biotechnology (MicroB3)**

With a budget of 9 Mio Euro for 4 years (2012–2015) Micro B3 will develop innovative bioinformatics approaches and a legal framework to make large-scale data on marine viral, bacteria, archaeal and protists genomes and metagenomes accessible for marine ecosystems biology and to define new targets for biotechnological applications.

Micro B3 will build upon a highly interdisciplinary consortium of 32 academic and industrial partners, comprising world-leading experts in bioinformatics, computer science, biology, ecology, oceanography, bio-prospecting and biotechnology, as well as legal aspects.

The work package focussing on marine biotechnology will deal with the exploration of integrated datasets looking at genes of unknown functions, as well as biosynthetic gene clusters in marine organisms and new genes from metagenomes. Laboratory experiments will be used to further confirm functions through pre-enrichments, functional screening of large-insert libraries, micro-colonies and micro-cultures as well as heterologous expression and bioassays.

The work package on intellectual property rights will develop documents and run workshops concentrating on

- Model agreements for pre-competitive access to microbial materials and exchange of materials and data within researchers.
- IP model agreements for pre-competitive access to large-scale microbial genomic research databases and
- Outreach workshop on topics like IPR, scientific potential, biotech options, environmental impact to discuss application and protection options with stakeholders from regional, national and international policy-making bodies.

Finally the work-package on dissemination, training and outreach will address the timely dissemination of relevant information via course, workshops and a programme of outreach to an interested public. Three expert workshops with industrial sector including SMEs as well as stakeholder workshops with policy makers & advisors in field of biodiversity protection are planned.

Another aspect of this package is capacity building through training of the next generation of scientists. For more detail including registration to the workshops and courses, follow our tweet and visit the website [www.microb3.eu](http://www.microb3.eu)

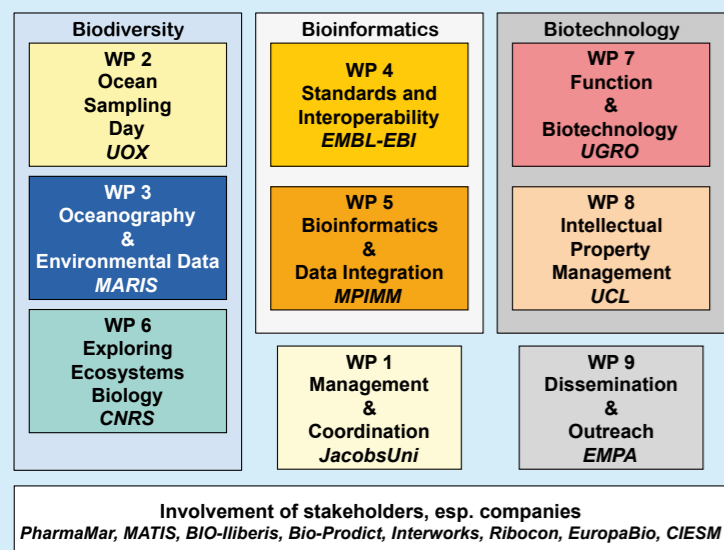


Figure 3: Work package structure of Micro B3, showing also the three pillars biodiversity, bioinformatics and biotechnology

**Analysis of cell powder from *in vitro* cultured fish cells**

Marina Gebert | Fraunhofer Research Institution for Marine Biotechnology (EMB), Germany

Seafood is considered to be important for a healthy diet, as it contains valuable proteins and high quality fats as related to the omega-6 to omega-3 ratio<sup>1</sup>. Additionally, the food market provides us with omega-3 enhanced food, in particular with functional food. The sources of highly unsaturated fatty acids are exclusively derived from the marine ecosystem. Beside algal sources of omega-3 fatty acids, predominantly cold water marine fish - especially oily species such as tuna, herring, trout or salmon - contain large amounts of highly unsaturated omega-3 fatty acids. The most important highly unsaturated fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These have been proven to be beneficial for human health.

(green bars) which are not supplemented by the cell culture medium (purple bars).

Encouraged by these findings, we tried to optimize the content of omega-3 fatty acids in the freeze dried fish cells. An increase of the total amount of EPA and DHA could be achieved by the addition of  $\alpha$ -linolenic acid (ALA) to the cell culture medium and the subsequent reduction of cell culture temperature. In Figure 2 we compare the fish cells shown in figure 1 with the optimized culture (orange bars). The uptake of ALA was enhanced 17 fold and the content of EPA and DHA increased 17.3x and 2.5x, respectively.

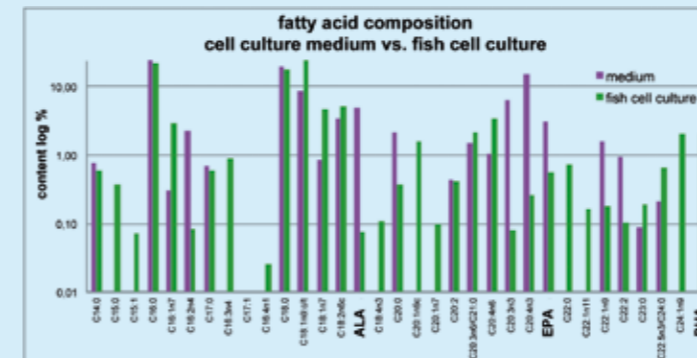


Figure 1: Fatty acid composition of freeze dried fish cells (green bars) compared with the cell culture medium used during *in vitro* cultivation (purple bars) in percent of total fat.

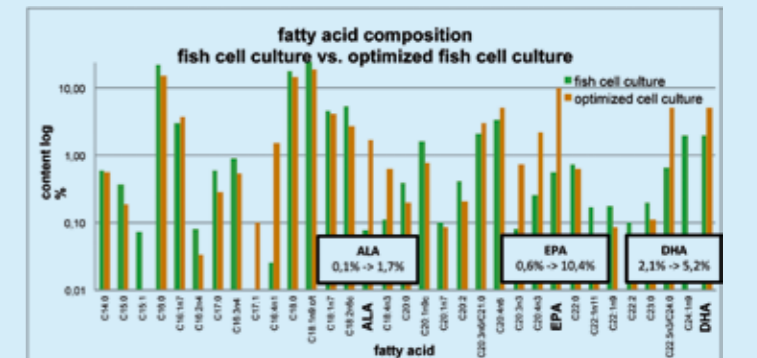


Figure 2: Fatty acid compositions of freeze dried fish cells. Untreated fish cells (green bars) compared with fish cells treated with  $\alpha$ -linolenic acid (ALA) and subsequent reduction of the cell culture temperature (orange bars) in percent total fat.

However, natural fish sources face two major problems: fish stocks stagnate or continuously decrease and heavy metals as well as lipophilic toxins like PCBs and PAHs tend to accumulate in fat tissue of fishes along the food chain. These lipophilic components, which are harmful to human health, are extracted along with fatty acids and lower their quality. Aquaculture, which is the strongest growing agricultural sector since the late 1970s, is using aquafeed containing fish meal and fish oil. These are as well produced from marine resources, thus putting even more pressure on the ecosystem ocean. Already in 2006, 56% of fish meal and 87% of fish oil was exclusively used for aquafeed production<sup>2</sup>. As aquaculture industry continues to grow, the exploitation of novel sources for proteins and omega-3 fatty acids far from marine ecosystems is ever gaining more importance.

However, an industrial production is not yet feasible, as *in vitro* culture of large quantities of anchorage dependent cells is currently cost-intensive. A scale-up of biomass production, as accomplished e.g. in bioreactors (Figure 3), faces some important challenges: (i) the discovery of appropriate carriers or scaffolds for cell growth, (ii) the achievement of high expansion rates for cells and (iii) the reduction of costs, mainly through mass production and enhanced technology. Resolving these problems in future years will enable access to fish derived products under controlled conditions, without contaminants and with low ecological damage.

We investigated the ability of a highly proliferative *in vitro* fish cell culture from Sturgeon larvae (*Acipenser oxyrinchus*) established in our laboratory<sup>3</sup>, which can be converted into “fish cell meal”, to produce nutraceutical grade omega-3 fatty acids. We found that these cells convert short chain polyunsaturated fatty acids into long chain highly unsaturated fatty acids during cell cultivation. Figure 1 shows the comparison of fatty acid compositions from the cell culture medium and the *in vitro* cell culture after harvesting and freeze drying. It is obvious that some fatty acids, amongst them DHA, are present in the freeze dried cells

**References**

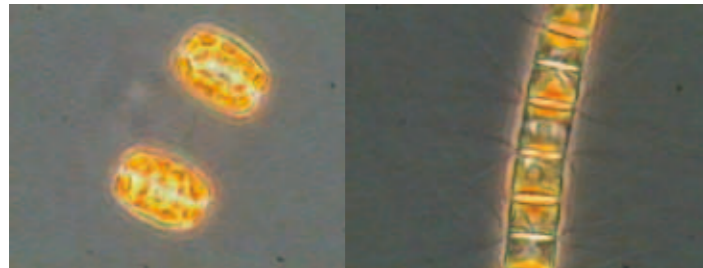
- <sup>1</sup> Larsen, R., Eilertsen, K.-E. Elvevoll, O. 2011. Health benefits of marine foods and ingredients. *Biotechnology Advances* 29 (5), pp. 508–518.
- <sup>2</sup> FAO, 2008. The state of world fisheries and aquaculture. FAO Fisheries and Aquaculture Department, FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS Rome, ISBN 978-92-5-106029-2.
- <sup>3</sup> Grunow, B., Noglick, S., Kruse, C., Gebert, M. Isolation of cells from Atlantic sturgeon *Acipenser oxyrinchus* and optimization of culture conditions. *Aquatic Biology* 14, pp. 67–75.

## Production of omega-3 fatty acids by Baltic Sea algae

**Kristian Spilling, Jukka Seppälä, Niklas Virkkala, Susanna Nenonen, Elina Salo, Katarina Natunen & Timo Tamminen | Finnish Environment Institute – SYKE, Finland**  
**Dagmar Enss & Heiko Rischer | VTT, Technical Research Centre of Finland, Finland**

Algae can contain high amounts of essential fatty acids (EFAs) that organisms at other trophic levels have limited abilities to synthesize. This has led to a lot of attention to algae as a potential feed for aquaculture and as raw material for the nutraceutical industry.

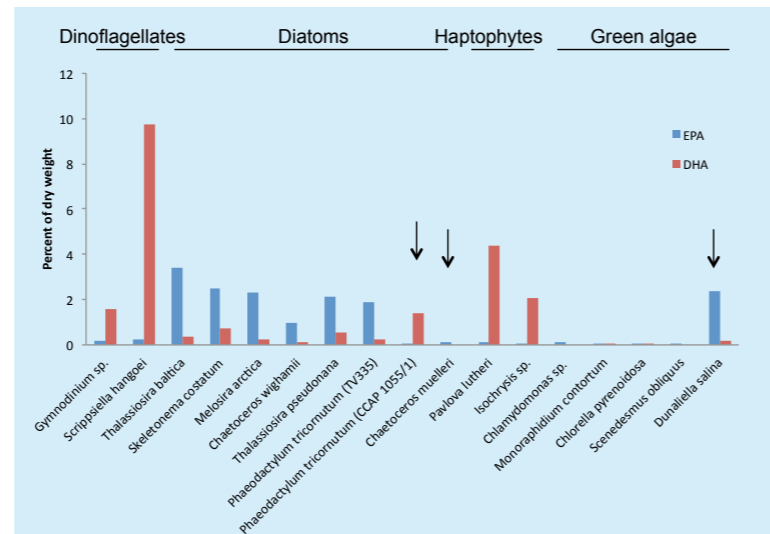
Omega-3 fatty acids, in particular Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) have known health benefits to humans and these fatty acids are produced in various amounts by algae. In order to find what algae would be best suitable for EPA and DHA production, we screened 20 different algal species, belonging to 4 different phyla, for their fatty acid composition in both exponential and stationary (N-limited) growth phase.



Two examples of Baltic Sea diatoms: *Thalassiosira baltica* (left) and *Chaetoceros wighamii* (right)



The cultures were grown in 8L polycarbonate bottles placed in water baths to ensure constant temperature. The culture medium was F/2, prepared from artificial seawater and inorganic nutrient stocks. Cultures were grown at 100 μmol photons m<sup>-2</sup> s<sup>-1</sup> in a 16h light, 8h dark cycle. Cultures were continuously aerated with sterile air pumped through a 0.2 μm filter.



**Figure 1:** Percent of EPA and DHA of dry weight of different taxa of algae marked with the horizontal bars on top. The samples were taken from N starved cultures. The vertical arrows denote marine species that were cultivated in full salinity water (35 PSU), all the other species originated from the Baltic Sea and was cultivated at lower salinity (6 PSU).

Diatoms contained the highest EPA concentrations whereas dinoflagellates and haptophytes were high in DHA (Fig. 1). Green algae and cyanobacteria contained very little of either EPA or DHA (with the exception of *Dunaliella salina* that contained some EPA). Generally, both EPA and DHA content increased in stationary growth phase. The highest EPA producer was *Thalassiosira baltica* (3.4% of DW) and most DHA was found in *Scrippsiella hangoei* (9.7% of DW).

In a separate experiment the effect of different nutrient limitation (N and P limitation) was studied in the diatom *Phaeodactylum tricornutum* and the haptophyte *Isochrysis* sp. The total lipid concentration was highest during N limitation; however, the EPA content in *P. tricornutum* and DHA content in *Isochrysis* sp. was highest during P limitation, 1.7% EPA and 1.7% DHA of DW respectively.

The results suggest that some algae can contain high amounts of omega-3 fatty acids (up to 10% of DW), but the production is highly phyla- and species-specific. The main omega-3 fatty acids produced was either EPA or DHA, but not at the same time. Different stress situations (e.g. nutrient limitation) increased the overall omega-3 concentration, but species specific optimizing should be carried out before any production starts.

## Healthy appetite for Algae: Milk products containing algae

**Marie Shrestha | ttz Bremerhaven, Food Technology & Bioprocess Engineering, Bremerhaven, Germany**

In Asia, algae are well-known food products since decades. In Germany food products based on algae are rare<sup>1</sup>: algae are primarily used as food additives such as gelling agents (alginate, agar and carrageen) or in pharmaceutical and cosmetic applications<sup>2</sup>. Algae are rich in vitamins, minerals, trace elements and fibre and contain low fat. The raising popularity of Asian food products in Europe is contributing to increase consumption of food products containing algae and at the same time contribute to a general improvement in health. In the current study curd and cream cheese products were enriched with brown algae for people with nutritive deficiencies<sup>3</sup>.

Germany is a deficient country for iodine intake and supplementation of iodine therefore needs to take place through enriched food products: iodized salt is a well know example. However whilst iodine deficiency interferes with the thyroid function, too high iodine consumption also has harmful health effects. The recommended daily allowance is 0,15 to 0,20 mg iodine per day. The maximum daily intake is 0,50 mg . A thorough formulation of dairy products with algae was therefore necessary.

Young *Laminaria saccharina* were purchased from an aquaculture farm in the North Sea (Sylter Algenfarm GmbH & Co.KG, List, Germany) in June and August 2009. The macroalgae were dried under mild conditions, homogenized and incorporated in dairy recipes to have a positive influence on the taste, texture and appearance of the products. The drying process decreased the vitamin C and polyphenol content but had no influence on the content of iodine. To efficiently reduce the iodine content the algae were immersed in boiling water for one minute.

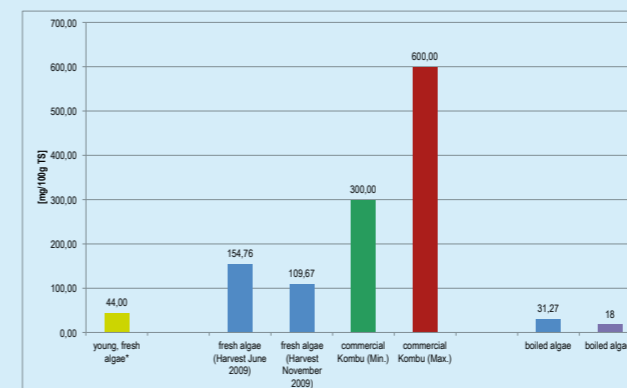
Algae were used both in powder form to imitate the granulation of pepper and in flake form to imitate fresh herbs. The sensory acceptance threshold was investigated to determine the maximum quantity of algae that can be added to cream cheese without impacting negatively the sensorial properties of the product. The addition of algae did not influence significantly texture, particle size and viscosity of the cream cheese recipes. The iodine content of the algae-rich cream cheese products was monitored: A maximum of 500g algae-rich cream cheese can be ingested per day without risk for health.

Consumers' acceptance and willingness to buy were determined: 60 men and women between 20 and 65 years of age rated cream cheese products containing algae in a blind test. Three cream cheese recipes were prepared with and without algae: salmon/herbs indish spices and wild garlic. The acceptance towards the innovative algae food products was good.

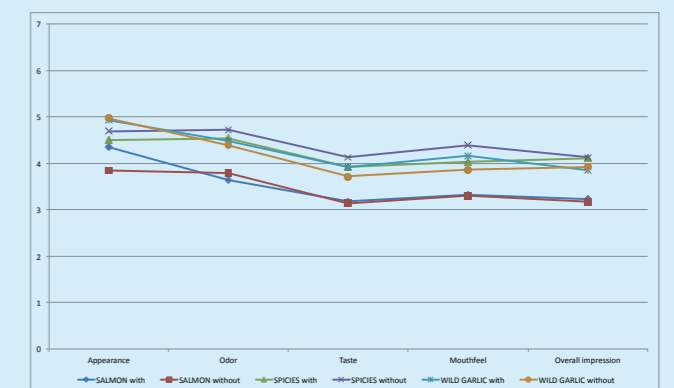
The optimisation of drying and grinding of young macro algae from the North Sea permits the standardised Production of algae-rich dairy products as prevention against nutritional deficiencies and as contribution towards more taste diversity.

### References

- Nisizawa, K. (2002). Seaweeds Kaisei bountiful harvest of the seas, sustenance of health and well being by preventing common life – style related diseases. World Seaweed Resources. An Authoritative Reference System. DVD-ROM. Ed. By A.T. Critchley, M. Ohn.
- Kroeger, V. (2007). Die ernährungsphysiologische Bedeutung von Algen für die Ernährung des Menschen und ihre Nutzung als Lebensmittel in der Zukunft. Hamburg: Diplomarbeit, Hochschule für angewandte Wissenschaften Hamburg.
- Project ALGAEFOOD: Development of drying and grinding processes of young brown algae for the production of algae-rich dairy products for prevention of nutritional deficiency; funded by the German federal ministry of economics and technology
- BMELV, B. f. (2007). BMELV, Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz. Ausreichende Jodversorgung – Wichtig für eine gesunde Entwicklung: <http://www.bmelv.de/SharedDocs/Standardartikel/Ernaehrung/GesundeErnaehrung/Ernaehrungsforschung/Jodversorgung.html>
- BfR, B. f. (2004). BfR, Bundesinstitut für Risikobewertung: Gesundheitliche Risiken durch zu hohen Jodgehalt in getrockneten Algen, Aktualisierte Stellungnahme Nr. 026/2007 vom 22. Juni 2004, S. 2: [http://www.bfr.bund.de/cm/208/gesundheitsliche\\_risiken\\_durch\\_zu\\_hohen\\_jodgehalt\\_in\\_getrockneten\\_algen.pdf](http://www.bfr.bund.de/cm/208/gesundheitsliche_risiken_durch_zu_hohen_jodgehalt_in_getrockneten_algen.pdf)



**Figure 2:** Influence of treatment of algae on the iodine content (claimed by the supplier)



**Figure 3:** Acceptance profile (from 1 very bad to 7 very good)

## From sustainably cultivated seaweed to certified organic cosmetics – The brand “Oceanwell” as a success story for a value-added chain originating from ecologically farmed marine resources

Inez Linke | oceanBASIS GmbH, Germany

The company oceanBASIS cultivates the sugarkelp *Saccharina latissima* in an integrative sustainable aquaculture facility in the Kiel Fjord. The company is specialised in extracting natural substances from marine organisms and develops natural products for health and beauty such as cosmetics.

### Algae farm

Seasonally marine biologists of oceanBASIS cultivate young algae sporophytes on seeding ropes in the laboratory. After about 8 weeks they are launched in the openwater farm, which is or-



ganic certified according to European organic standards. The seedlings grow about half a year in fresh seawater until they become harvested in early summer.

### Extract

After harvesting the fresh algae are fermented to an alcoholic extract rich in active marine ingredients such as minerals, iodine, special algae sugars and polyphenols.

### Cosmetics

Based on this unique extract and natural seawater oceanBASIS developed the NaTrue certified organic cosmetic brand “Oceanwell” with 10 products for face and body skin care. Nationally Oceanwell is distributed via an online shop, perfumeries, natural cosmetics shops, spas and hotels. Export has started recently first to China, now to the USA.

Clinical and Dermatest studies revealed that Oceanwell has a beneficial skin-compatible effect. Thus it is suitable especially for dry and sensitive skin. Moreover, Oceanwell moisturizes, regenerates, energizes and protects the skin in a natural way.

After 7 years of research oceanBASIS invented a new cosmetic ingredient, oceanic collagen, extracted from jellyfish. Studies revealed unique high waterbinding capacity. The organically certified cosmetic ProAge Line “OceanCollagen” was developed specially for demanding skin. Dermatest studies showed that wrinkles depth is reduced significantly (-25%) by the OceanCollagen face cream.

## Sulfated polysaccharides of *Delesseria sanguinea* from the artificial reef Nienhagen

Susanne Alban, Niels Grünewald, Juliane Grimm & Inken Groth | Pharmaceutical Institute, Christian Albrechts University of Kiel, Germany

In 2003, a large-scale artificial reef was established in the Baltic Sea close to Nienhagen (Germany) in order to increase the local fishery resources. In addition to an increase of the fishing value, the reef structures turned out to be abundantly colonized by algae, predominantly by the perennial red macroalga *Delesseria sanguinea* (Hudson) Lamouroux (D.s.). Compared with D.s. occurring in North Atlantic and North Sea, D.s. from the reef has the advantage to be scarcely contaminated with epiphytes. Since algae-based products are of growing interest, the sub-project ‘Evaluation of the economic applicability of the Baltic Sea red alga *Delesseria sanguinea*’ was initiated.

D.s. turned out to contain substantial amounts of a non-gelling sulfated polysaccharides consisting of a homogenous fraction of branched sulfated xylogalactans (D.s.-SP) (galactose: xylose ~5.4)<sup>1</sup> (Figure 1).

D.s.-SP has a pharmacological profile indicating anti-inflammatory and anti-skin aging potencies<sup>1,2</sup>. It exhibits inhibitory effects on extra-cellular matrix degrading enzymes like PMN-elastase, hyaluronidase, heparanase, collagenase (MMP-1) as well as on the alternative and classical complement activation, the adhesion of neutrophils and tumor cells to P-selectin and the cytokine release from LPS-activated monocytes. Further, D.s.-SP is neither cytotoxic nor phototoxic and showed potent free radical scavenging activity by inhibiting the generation of reactive oxygen species in UV-irradiated human keratinocytes (HaCaT).

Comparison of the activity profiles of D.s.-SP with those of heparin, the prime example of a bioactive sulfated polysaccharide,

and of PS3, a semisynthetic  $\beta$ -1,3-glucan sulfate with proven in vivo anti-inflammatory potency<sup>2,3</sup>, revealed the following<sup>4</sup>:

1. both D.s.-SP and PS3 have only moderate anticoagulant activity and thus a considerably reduced bleeding risk;
2. most of the other activities of both D.s.-SP and PS3 are considerably stronger;
3. D.s.-SP and PS3 have a similar, but not identical activity profile (Figure 2).

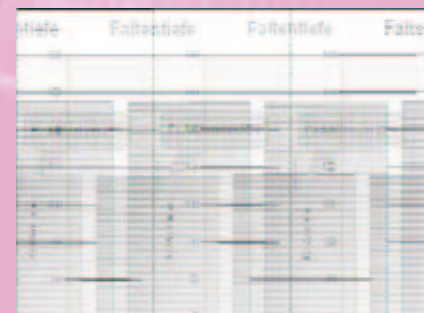
Crucial for an economic use is the availability of adequate amounts of D.s.-SP with reproducible high quality. For this, almost 230 D.s.-SP batches were isolated from 30 D.s. batches harvested over the period of 6 years, analyzed and tested. The results impressively illustrate that “the process defines the product” as known for biological compounds. By evaluating numerous parameters potentially influencing the quality of D.s.-SP and successive optimization of the process, the D.s.-SP can now be isolated in reproducibly high quality by using a specific extraction procedure (Figure 3). This is an important difference of the D.s.-SP compared to other algae-derived extracts and compounds (e.g. fucoidans)<sup>5</sup>. The average yields of D.s.-SP originally amounted to  $11.9 \pm 4.4\%$  in dependence on the harvest time of the algae [6] (Figure 4), but could meanwhile be increased to about 20%. By applying the established procedure, a scaling-up experiment (100 kg fresh D.s.) led to D.s.-SP of identical quality as obtained in our lab.

*Acknowledgments: This project is financed by the EU (FIAP/EFF) and the LFALF Mecklenburg-Vorpommern.*

## OceanCollagen ProAge Line

### Face Cream

„Most Innovative Natural Product 2012“



## Oceanwell Basic.Line – marine and pure

- moisturize
- regenerate
- protect
- revitalize



*Compared to red algae from the North Atlantic and North Sea, Delesseria sanguinea from the Baltic reef has the advantage of scarcely being contaminated with epiphytes.*

**D.s.-SP – a homogeneous fraction of branched, sulfated xylogalactans with 1 sulfate group per disaccharide**

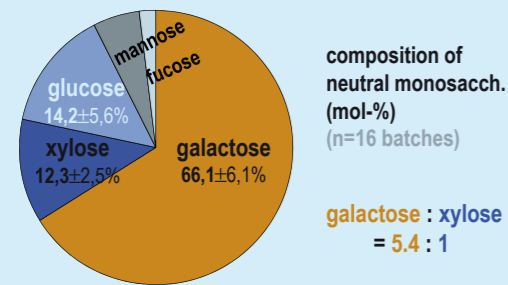


Figure 1

**Pharmacological Profile of D.s.-SP in comparison with that of heparin and PS3**

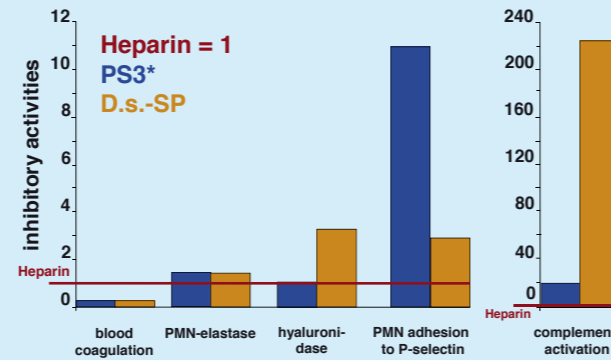


Figure 2

**Influence of harvest time of D.s. e.g. on PMN-elastase inhibition by D.s.-SP**

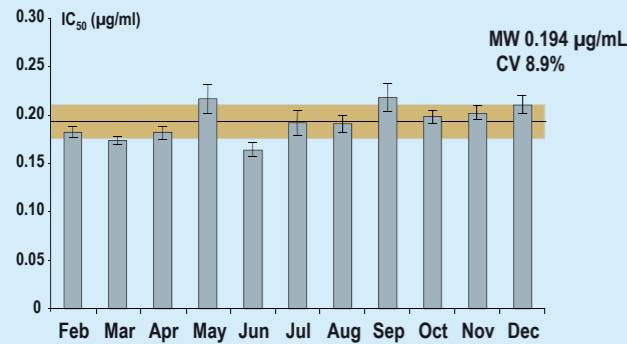


Figure 3

**Influence of harvest time of D.s. on yield (% related to dry mass) of D.s.-SP**

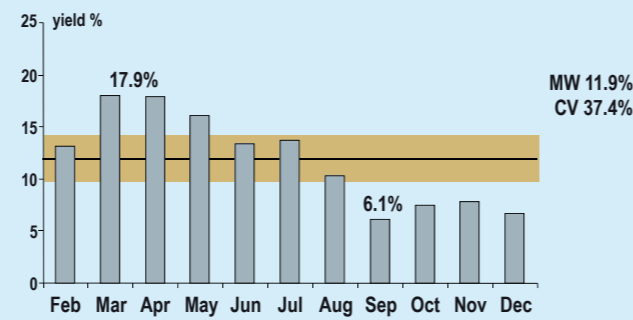


Figure 4

**References**

- Grünwald N, Alban S: Optimized and standardized isolation and structural characterization of anti-inflammatory sulfated polysaccharides from the red alga *Delesseria sanguinea* (Hudson) Lamouroux (Ceramiales, Delesseriaceae). *Biomacromolecules* 10; 2009: 2998–3008.
- Alban S, Ludwig RJ, Bendas G, Schön MP, Oostingh GJ, Radeke HH, Fritzsche J, Pfeilschifter J, Kaufmann R, Boehncke WH: PS3, a semisynthetic beta-1,3-glucan sulfate, diminishes contact hypersensitivity responses through inhibition of L- and P-selectin functions. *J Invest Dermatol.* 2009; 129:1192–1202.
- Yvin J-C, Alban S, Franz G: Anti-inflammatory and healing medicine based on laminarin sulphate. *PCT Int. Appl. WO 0236132-A1, Fr 281613-B1, US 7008931-B2* (2006).
- Groth I, Grünwald N, Alban S: Pharmacological profiles of animal- and nonanimal-derived sulfated polysaccharides--comparison of unfractionated heparin, the semisynthetic glucan sulfate PS3, and the sulfated polysaccharide fraction isolated from *Delesseria sanguinea*. *Glycobiology* 19(2009) 408–417.
- Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI, Usov AI, Ustyuzhanina NE, Grachev AA, Sanderson CJ, Kelly M, Rabinovich GA, Iacobelli S, Nifantiev NE. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology.* 2007;17: 541–552.
- Grünwald N, Groth I, Alban S. Evaluation of seasonal variations of the structure and anti-inflammatory activity of sulfated polysaccharides extracted from the red alga *Delesseria sanguinea* (Hudson) Lamouroux (Ceramiales, Delesseriaceae). *Biomacromolecules* 10( 5) (2009) 1155–1162.

**From marine biodiversity to innovative drugs. The experience of PharmaMar**

Fernando de la Calle | PharmaMar S.A., Spain

Traditionally, higher plants and terrestrial microorganisms have proven to be the richest sources of natural drugs. However, we are living in a planet of oceans. The marine ecosystem covers more than 70% of the Earth's surface but represents 95% of the biosphere. The first living organisms appeared in the sea more than 3,500 million years ago and evolutionary development has equipped many marine organisms with the appropriate mechanisms to survive, developing exquisitely complex biological and chemical mechanisms for defence, attack, signalization and other still unknown purposes. These biological capabilities are clearly revealed by their ability to biosynthesize and release potent chemical weapons that are active per se.

Such novel chemical structures often result in new modes of action and open up the potential of new ways to treat cancer and other diseases. The current scientific, academic and biotech-pharmaceutical industries have recognized this opportunity and thousands of bioactive compounds are being discovered and some of them are being testing in clinical trials, mainly in oncology. But the conversion of a bioactive molecule into a medicine is a long and risky process. It involves astronomic investment for developing the identification and validation of new targets, drug discovery, medicinal chemistry and drug delivery, apart from ensuring the future supply, where chemical synthesis and biotechnology are the preferred sources for manufacturing.

This was the case of the PharmaMar pipeline compounds as the anticancer medicine Yondelis® (PharmaMar, Spain) isolated from a marine tunicate *Ecteinascidia turbinata*, that is currently manufactured using a hemi-synthetic process or the total synthesis for manufacturing Aplidin®, peptide isolated from the tunicate *Aplidium albicans*.

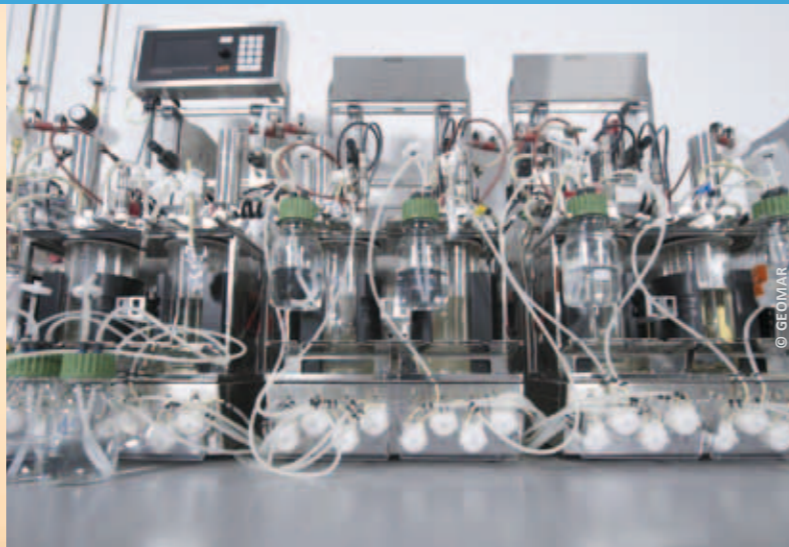
Natural collections are typically used only for drug discovery purposes, where only small amounts (milligram scale) of the pure compound are enough to elucidate the chemical structure and the biological activity.

The marine bioprospection, involving the study of extracts from invertebrates, microorganisms and recently, exploring DNA from uncultivable organisms, involves large amount of investment and is a long and risky process with a low chance of finally reaching the market. Today, it is essential to innovate efficiently for increasing the probability of developing new scaffolds, reducing time and costs.

The classical view of marine biotechnology has been radically changed with the advent of molecular tools, mainly due to innovative methods for detecting uncultured microorganisms and the massive and efficiency use of DNA sequencing technologies and powerful computation features to define interesting gene cluster and prediction of core structures formed by them.

THERAPEUTIC AREA	INDICATION	CLINICAL DEVELOPMENT				
		PHASE I	PHASE II	PHASE III	REGULATORY	MARKET
<b>ONCOLOGY</b>						
<b>Yondelis® Trabectedin</b>	Soft Tissue Sarcoma(STS) – 2nd/3rd line					
	Relapsed Ovarian cancer (Yondelis+Doxil)– 2nd/3rd line					
	STS with Translocations – 1st line					
<b>Aplidin® Plitidepsin</b>	Multiple Myeloma					
	T-cell lymphoma - non cutaneous					
<b>Zalypsis®</b>	Multiple Myeloma					
	Ewing Sarcoma					
<b>PM01183</b>	Pancreatic cancer					
	Ovarian Cancer (Resistant)					
	Breast cancer					
	Leukemia					
	Combination Studies					
<b>PM060184</b>	Solid tumours					

**PharmaMar's clinical pipeline**



*The conversion of a bioactive molecule into a medicine is a long and risky process involving astronomic investments.*



## Marine microorganisms – a promising source for novel therapeutics

Sabine Mundt, Wolf-Dieter Jülich & Ulrike Lindequist | Institute of Marine Biotechnology e.V. and Institute of Pharmacy, Ernst Moritz Arndt University Greifswald, Germany  
Gerold Lukowski | Institute of Marine Biotechnology e.V., Germany

Marine natural products chemistry was born in the 1960s and entered adolescence during the 1980s. The number of natural products discovered annually increased from less than 100 to a level of about 500 products per year in the late 1990s. In 2010 more than 1,000 new structures with a wide range of bioactivities have been published. Worldwide marine organisms, especially more and more microorganisms such as fungi, bacteria and cyanobacteria, are recognized as sources for novel natural products.<sup>1-3</sup>

The focus of research in our group is the isolation and characterization of active compounds produced by marine fungi and cyanobacteria (see below pictures). Based on a culture collection of more than 400 strains isolated from different sources (Baltic Sea, North Sea, Red Sea, South China Sea, Vietnam and South Sulawesi, Indonesia) organisms are cultured in different volumes from 100 ml until 50 l (see pictures on right page). Extracts are prepared from biomass and cultivation medium with solvents of different polarity and tested in-vitro for activity against Gram-positive and Gram-negative bacteria and pathogenic fungi, for cytotoxic activity to tumor cells and for activity against viruses such as influenza virus and herpes simplex virus.

Active substances are isolated bioassay-guided by column chromatography including HPLC and the structures are elucidated by NMR and MS.

Fractionation of the ethyl acetate extract of the cultivation medium of the ascomycetes fungus *Corollospora maritima*, isolated from driftwood collected near Helgoland, resulted in the identification of the natural phthalide derivative corollosporin A. The compound inhibits the growth of Gram-negative and Gram-positive bacteria.<sup>4</sup> Two benzofuranoids, deuteromycol A and B, have been isolated from the ethanol extract of the marine-derived strain MF 003 (Deuteromycete), obtained from Red Sea mangrove drift wood and seem to be responsible for the activity of the ethanol extract to different *Staphylococcus aureus* strains including MRSA.<sup>5</sup> Fungal strains from Indonesian marine habitats were isolated and the ethyl acetate extracts tested for antifungal activity to plant pathogenic fungus *Cladosporium cucumerinum*. From the culture broth of the endophytic *Daldania eschscholzii* strain isolated from red alga *Gracilaria* sp. SGR-1 the lactone heliascolid C was isolated as the active compound with specific antifungal effects.<sup>6</sup> Six new naphthalenone derivatives, Balticals 1–6, from an ascomycetes fungus, isolated from drift-

Now, the concept of biological diversity is based on an enormous universe of DNA sequences, where the majority of the life forms cannot be cultivated in the laboratory. In addition, thousands of whole microbial genomes are being sequenced and a large number of silent genes related to secondary metabolisms are appearing, potentially responsible of the biosynthesis of new chemical scaffolds which could be expressed by heterologous expressions in the close future. This is great news for the scientific community (and, of course, for the biotechnology sector). It opens up the possibility of analyzing all this genomic content as potential genes (mainly Polyketide Syntheses and Non Ribosomal Peptide Synthetases) able to produce innovative pharmaceutical compounds and enzymes.

As example of the potential of genomic mining are the cases of the recently published hypothetical biosynthetic pathway of trabectedin (Yondelis) by pyrosequencing and informatic annotation of NRPS belonging to associated microbial symbionts in the ascidia *E.turbinata*<sup>1</sup> or the description of the complete gene

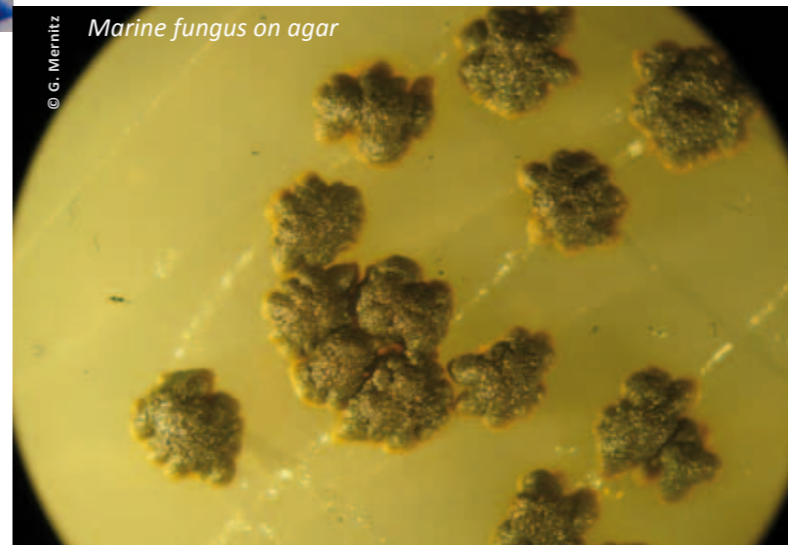
cluster PKS/NRPS of didemnins, chemical family of Aplidin, in a mega plasmid in a free living alpha proteobacteria<sup>2</sup>. This family class of metabolites had been described only in marine ascidians.

Today we are beginning to explore this metagenomic approach. Soon, the number of new compounds with applications in human health is expected to increase dramatically as a result of using innovative genetic technology.

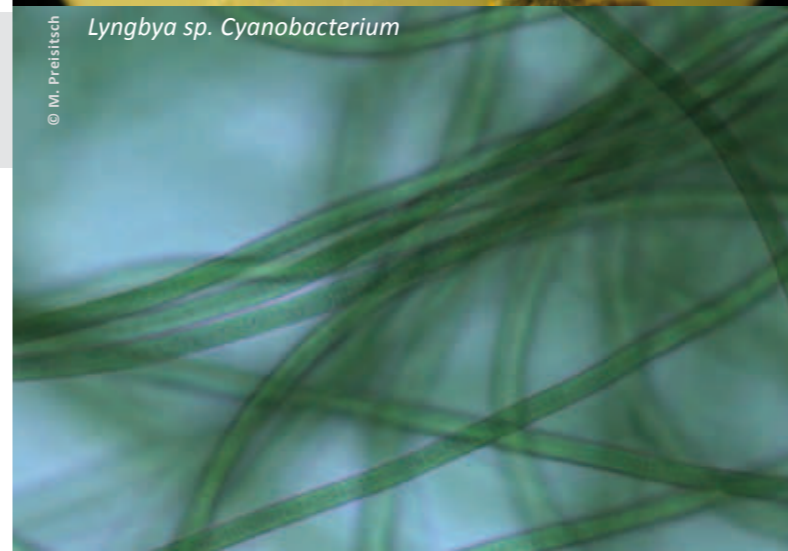
PharmaMar (Zeltia group), a Spanish biotechnology company, devoted on the research and development of marine natural products for the treatments of human cancers, is developing an ambitious drug discovery programme using marine samples.

### References

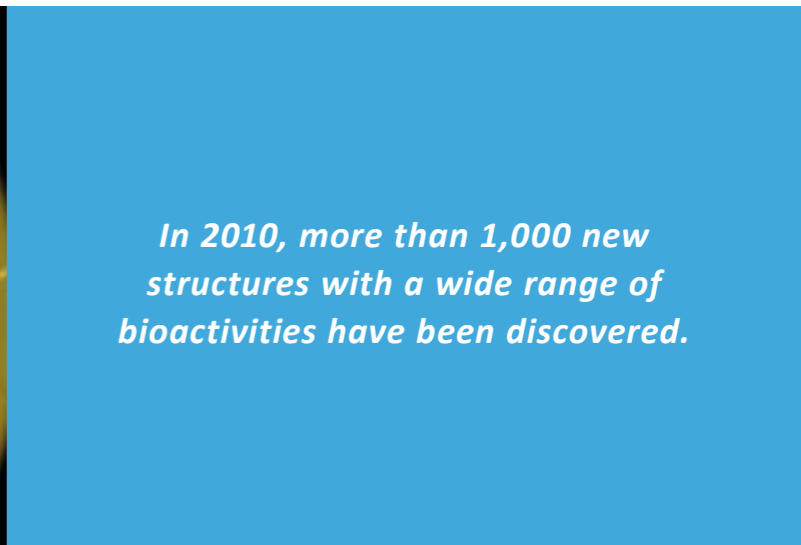
- <sup>1</sup> Rath et al.; ACS Chem. Biol. 2011, 6, 1244–1256.
- <sup>2</sup> Xu et al.; JACS, DOI: 10.1021/ja301735a, April, 2nd, 2012.



Marine fungus on agar



Lyngbya sp. Cyanobacterium



Bio33-Maresome®

*In 2010, more than 1,000 new structures with a wide range of bioactivities have been discovered.*

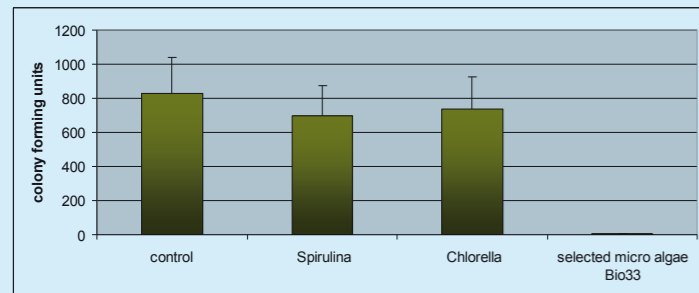


wood, collected from the Greifswalder Bodden, displayed inhibitory activity against influenza virus A and herpes simplex virus.<sup>7</sup>

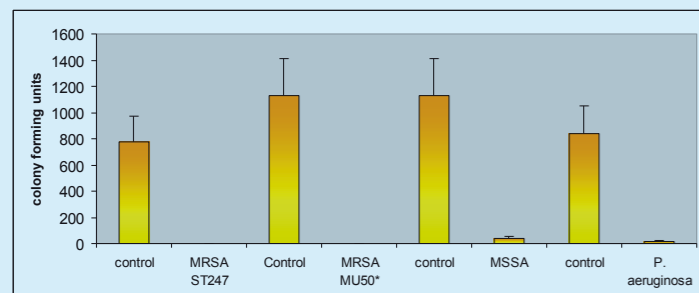
From cultivated Vietnamese cyanobacteria belonging to the Genus *Nostoc* polyketides with strong cytotoxic activity to tumor cell lines and moderate antibacterial effects were isolated. It was shown, that the compounds possess stronger cytotoxic activity against tumor cells than against non transformed cells.<sup>8</sup> A *Lyngbya* strain was identified as the source of antibacterial and cytotoxic active cyclic undecapeptides, lyngbyazothrins A–D<sup>9</sup>. The methanol-water extract prepared from the biomass of the *Anabaena* strain Bio 33, obtained from the Baltic Sea near island of Ruegen, exhibited specific activity against human pathogenic

yeasts and fungi, such as *Candida albicans*, *Aspergillus* and *Mucor* sp. Complex cyclic lipopeptides are responsible for antifungal activity; structure elucidation is in progress.

Besides the isolation of pure active compounds we use whole biomass of marine microorganisms to prepare microparticles by Maresome-technology.<sup>10</sup> It could be shown that microparticles from the biomass of Bio 33 called Bio33-Maresome® (see picture on left page) in-vitro inhibit dermal colonization of different MRSA strains and even of the VRSA MU50 strain (Fig. 1–2).<sup>11</sup> A prophylactic skin care with Bio33-Maresome® to prevent nosocomial infections has been developed. First in-vivo data have confirmed the in-vitro effects.



**Figure 1:** Influence of Maresome® prepared from biomass of Bio 33 (N=157), Spirulina and Chlorella (N=14) on colonization of MRSA (NES) in cow udder test (direct contamination after treatment with Maresome® ointment)<sup>11</sup>



**Figure 2:** Influence of Maresome® prepared from biomass of Bio 33 (N=54) on colonization of different MRSA strains and *Pseudomonas aeruginosa* in cow udder test (direct contamination after treatment with Maresome® ointment)

\*Vancomycin resistant MRSA Mu50

**References**

- Hu, G.-P. et al., *Mar. Drugs* 2011, 9, 514–525.
- Blunt, J.W. et al., *Nat. Prod. Rep.* 2012, 29, 144–222.
- Hill, R.T. et al., *Curr. Opin. Biotechnol.* 2010, 21, 777–779.
- Liberra, K. et al. *Pharmazie* 1998, 53: 578–581.
- Nawwar, M. et al., *Arch. Pharm. Res.* 2010, 33, 1729–1733.
- Tarman, K. et al., *Phytochem. Lett.* 2012, 5, 83–86.
- Shushni, M. et al., *Chem. Biodivers.* 2009, 6, 127–137.
- Bui, H. et al., *J. Nat. Prod.* 2007, 70, 499–503.
- Zainuddin, E. et al., *J. Nat. Prod.* 2009, 72, 1373–1378.
- Lukowski, G., Jülich, W.-D., Lindequist, U., Mundt, S., 2005, PCT/DE03/00747 bzw. EP1480661 05.01.2005.
- Lukowski, G. et al., *Skin Pharmacology and Physiology* 2008, 21, 98–105.
- Lukowski G. et al., *Kosmetische Medizin* 201031, 32–33.

**AAC – Algae Against Cancer: Preliminary data of a national research project**

**Marion Zenthoefer & L. Piker | CRM – Coastal Research & Management, Germany**  
**M. Peipp, M. Gramatzki, U. Geisen & H. Kalthoff | University Medical Center Schleswig-Holstein (UKSH), Germany**  
**Y. Weniger & S. Alban | Christian Albrechts University Kiel, Germany**  
**S. Hennig | Source BioScience Ltd., Germany**  
**M. Fuhrmann, F. Huber & R. Kirchhöfer | LipoFIT Analytic GmbH, Germany**

Based on previous studies, demonstrating significant inhibitory effects on proliferation of tumor cells triggered by various extracts of different macroalgae (Fig. 1), a national research network will now enlighten structure and mechanisms of action of potent ingredients of marine algae.

In the project “Algae Against Cancer” (AAC), funded by the Federal Ministry of Research and Education (BMBF), three private and three university partners investigate cellular responses

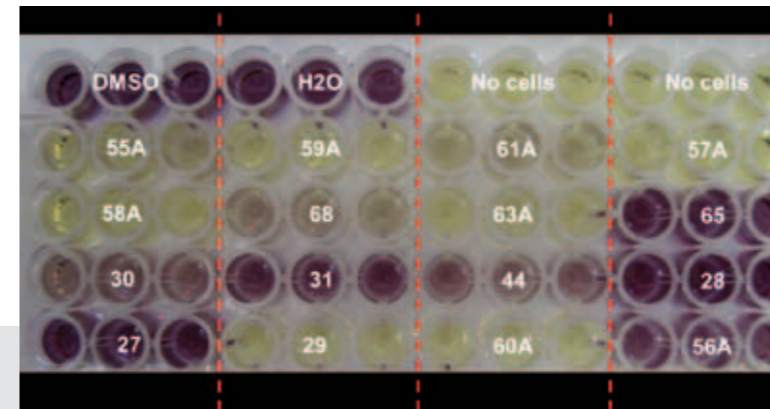
upon treatment with algae-derived extracts. Possible synergistic effects of a combined action of several substances will be analyzed with innovative methods of genomics and metabolome analysis.

Preliminary results from studies with different hematologic and solid tumor cell lines are presented showing patterns of inhibition of cell proliferation (Fig. 2). Some algae extracts inhibit also target enzymes like Elastase or Tyrosinkinase species.

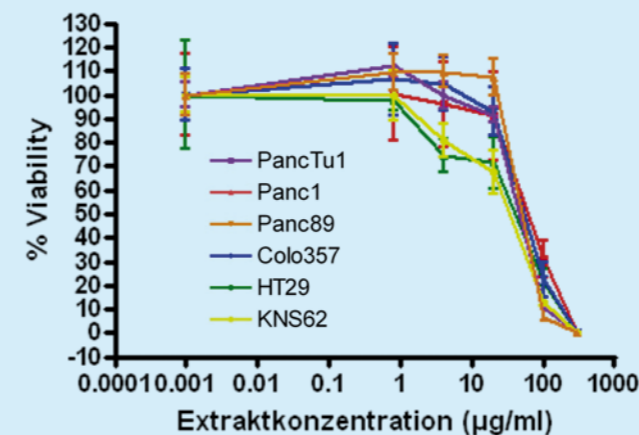
Furthermore, first alterations in gene expression profiles have been detected and will lead to the selection of target genes as objects for a detailed qPCR study.

Also, metabolic responses have been identified in terms of NMR analyses. The differences in the composition of metabolites in algae extract-treated and untreated tumor cells (Fig. 3) will result in a range of model components, which will give insight into metabolic changes and, thus, help to identify possible anti-cancer mechanisms.

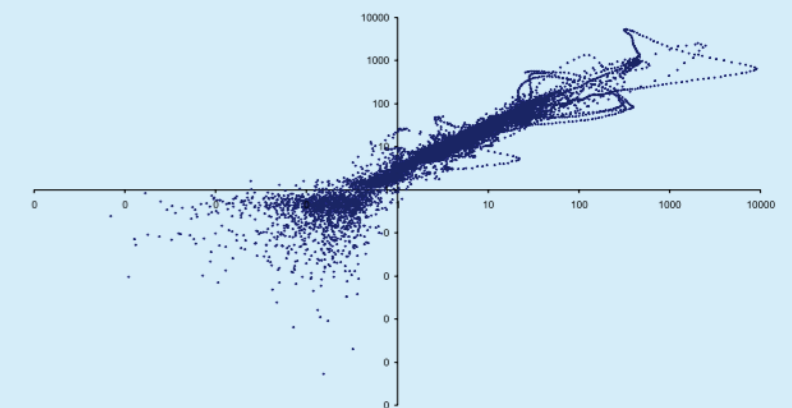
The first results suggest a real chance, i) for specifying substances that have not yet been described as cancer therapeutics, ii) for the identification of structural modifications of already known drugs leading to improved anti-tumor activity, and iii) for describing previously unknown mechanisms of action of known algal ingredients.



**Figure 1:** Effects of algae extracts (each number is representing a different extract) on the proliferation of human lymphoma cell line Raji. Dark wells: strong proliferation, pale yellow: low proliferation.



**Figure 2:** Cell viability in terms of a XTT-assay after incubation (72 h) of six solid tumor cell lines with an algae extract using different concentrations.



**Figure 3:** Comparison of the signals from two different NMR spectra (from solid tumor cells treated with an algae extract vs. not treated).

## Integrated analytical approaches towards marine natural products discovery

Thomas Ostenfeld Larsen, Maria Månsson & Kristian Fog Nielsen | Center for Microbial Biotechnology (CMB), Technical University of Denmark, Denmark

Charlotte Held Gotfredsen | Department of Chemistry, Technical University of Denmark, Denmark

Lone Gram | National Food Institute, Technical University of Denmark, Denmark

Per Juel Hansen | Marine Biological Section, University of Copenhagen, Denmark

Many strategies can be followed in discovering novel drugs from microbial sources. We have demonstrated that phenotypic based microbial taxonomy can be a successful path to microbial drug discovery<sup>1</sup>. This involves the use of multivariate methods for clustering and selection of talented strains for testing in various bioassays (e.g. antimicrobial, anticancer). The second step in the discovery process is **dereplication**, where we use *state-of-the-art* high resolution mass spectrometry (<1 ppm mass accuracy and accurate isotope pattern) in combination with comprehensive compound databases in order to ensure not to waste time isolating and elucidating the structures of already known compounds<sup>2</sup>. When likely unknown compounds have been identified an explorative solid-phase extraction approach<sup>3</sup> is applied for micro-scale fractionation on a set of different types of columns (RP, ion-exchange, size, NP), in order to determine more chemical properties of the bioactive(s). This further aids selection of a fast optimal purification strategy towards the pure bioactives prior to NMR characterization.

The presentation illustrated our integrated analytical approaches and highlighted some of the results that we have gained in a recent larger Danish biodiscovery project focusing on marine bacteria<sup>4-6</sup>, as well as some of our future challenges in determining the chemistry of microalgae and marine derived fungi.

### References

- <sup>1</sup> Larsen, T.O., Smedsgaard, J., Nielsen, K.F., Hansen, M.E. & Frisvad, J.C. 2005. Phenotypic taxonomy and metabolite profiling in microbial drug discovery. *Natural Product Reports*, 22, 672–695.
- <sup>2</sup> Nielsen, K.F., Månsson, M., Rank, J., Frisvad, J.C. & Larsen, T.O. (2011). Dereplication of microbial natural products by LC-DAD-TOFMS: Experiences gained from an inhouse database of 719 mycotoxins and fungal metabolites. *Journal of Natural Products*, 74, 2338–2348.
- <sup>3</sup> Månsson, M., Phipps, R.K., Gram, L., Munro, M.H.G., Larsen, T.O. & Nielsen, K.F. (2010). Explorative Solid-Phase Extraction (E-SPE) for accelerated microbial natural product discovery, dereplication and purification. *Journal of Natural Products*, 73, 1126–1132.
- <sup>4</sup> Månsson, M., Nielsen, A., Kjærulff, L., Gotfredsen, C.H., Wietz, M., Ingmer, H., Gram, L. & Larsen, T.O. (2011). Inhibition of virulence gene expression in *Staphylococcus aureus* by novel depsipeptides from a marine *Photobacterium*. *Marine Drugs*, 9, 2537–2552.
- <sup>5</sup> Månsson, M., Gram, L. & Larsen, T.O. (2011). Production of bioactive secondary metabolites by marine Vibrionaceae. *Marine Drugs*, 9, 1440–1468.
- <sup>6</sup> We acknowledge support from the Programme Committee for Food, Health and Welfare under the Danish Strategic Research Council.

## Aquapharm – Application of Blue Biotechnology for the discovery of novel pharmaceuticals

Andrew Mearns Spragg | Aquapharm Biodiscovery Ltd, European Centre for Marine Biotechnology, United Kingdom

We live on a Blue Planet: more than 70% of the Earth's surface is covered by oceans. Our economies and our health and wellbeing are critically dependant on the resources and services provided by the seas and oceans. Aquapharm Biodiscovery Ltd is an innovative discovery company pioneering the development of new pharmaceuticals and functional ingredients founded on the vast, untapped diversity of marine micro-organisms.

Over the past 20 years, the pharmaceutical industry has seen a decrease in discovery and success based on computational and combinatorial chemistry, and an increase in base costs imposed by increasing regulatory requirements and a higher rate of failures during the development process<sup>1</sup>. Couple this to the potential of structural, hence intellectual property overlap between libraries of synthetic compounds and the need for low-cost, scalable, and chemically unique new scaffolds, the pharmaceutical industry is starting to re-evaluate the potential of natural products to provide the industry with new drug leads.

Modern pharmaceutical drug discovery owes much to natural product research as more than 61% of small molecule chemical entity (NCE) drugs marketed between 1981 and 2002 originated from natural products.<sup>2,3</sup> For antimicrobial and anticancer therapies, the figures are 78% and 74% respectively. The majority of these products have been derived from so called 'secondary metabolites' of terrestrial microorganisms, however the rate of new chemistry discovered from land based organisms has slowed and there is growing recognition of the potential of the marine environment for the provision of new biological and chemical diversity.

Marine organisms are highly diverse and represent a unique source of genetic information and biochemical complexity, associated partly with a.) genetic diversity with for example fourteen endemic (unique) marine phyla compared with just one endemic terrestrial phylum, for example, and only perhaps 240,000 different organisms found so far by the Census of Marine Life out of an estimated 1.4–1.6 million - and partly with b.) ecological diversity - habitats range from beaches and mudflats to extreme ocean depths of 11 km, more than hundreds of metres deep in sediments, as well as the water in-between, all with enormous physical and chemical variability.<sup>4</sup>

Marine biodiversity is potentially exploitable in a wide range of economically important sectors. Large numbers of marine-origin molecules with structural novelty are now being described on monthly basis, which highlight the significant growth which has recently taken place in the screening and exploitation of marine genetic resources. More than 18,000 natural products and 4,900 patents have to date been derived from the genes of marine organisms, and the number of patents is increasing by 12% per annum. In the healthcare area, for example, there continue to be unmet needs in managing and treating cancer, chronic in-

flammatory diseases, metabolic dysfunctions and infectious diseases<sup>5</sup>. Currently there are around 15 marine natural products in various phases of clinical development, mainly in the oncology area, with more on the way and several products already on the market.

### Examples of marine natural products of pharmaceutical potential

Product	Disease indication	Compound origin (Organism)	Status
Prialt®	Neuropathic Pain	<i>Conus magus</i> (Mollusc)	Commercial
Yondelis®	Cancer	<i>Ecteinascidia turbinata</i> (Ascidian)	Commercial
Halaven®	Cancer	<i>Halichondria okadaei</i> (Sponge)	Commercial
Salinisporamide	Cancer	<i>Salinispora tropica</i> (actinomycete)	Phase I
Plinabulin	Cancer	<i>Aspergillus sp.</i> (Fungus)	Phase II
Pelorol	Inflammation	<i>Dactylospongia elegans</i> (Sponge)	Not Known

To support Aquapharm's own screening efforts to find novel chemistry, the Company has developed a proprietary drug discovery tool; SeaRch™; a novel method of accessing chemistry locked away in cryptic biosynthetic pathways within micro-organisms.

### SeaRch™

Due to the fundamental limitations of accessing the full range of chemical productivity produced from micro-organisms (marine or terrestrial) many of which are present in "cryptic" / or locked away biosynthetic pathways, Aquapharm has developed a novel approach of modulating the microbial metabolome to unlock the bio-synthetic potential of micro-organisms for the discovery of new compounds – many of which are capable of modulating hard to drug protein: protein drug targets. This is our SeaRch™

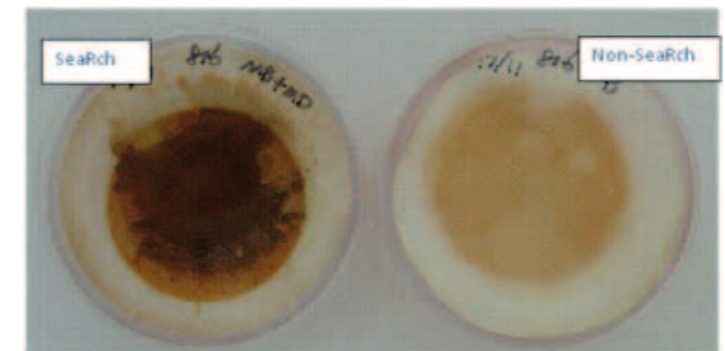
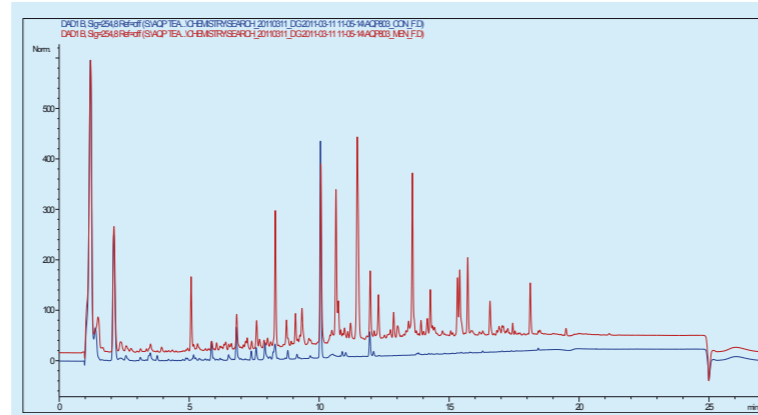


Figure 1: A marine microbial biofilm exposed to oxidative stress conditions (right) vs normal culture conditions (left)

platform which leverages IP initially filed in 2007 on techniques to achieve the reactivation of cryptic secondary metabolite pathways in micro-organisms via epigenetic remodeling in response to the induction of oxidative stress<sup>6</sup>.

Aquapharm has utilized SeaRch™ to screen 1,100 marine strains from its collection of over 9,500 marine isolates, for the discovery of novel antibiotic and anti-inflammatory compounds with our partners AMRI based near Seattle.

Preliminary data has demonstrated that this has been a great success and has yielded a substantial number of novel pharmacophoric scaffolds, many of which are below 500m/z and fall well within the Lipinski rules for drug like chemistry. Further screening of some of these novel drug-like compounds against a broad range of GPCRs, enzymes & ion channels has discovered chemistry that is highly potent and also highly specific against interesting and complex drug targets reading onto CNS & oncology.



**Figure 2:** Example of increased production of secondary metabolites by an Aquapharm microbial strain treated by SeaRch™ – HPLC profiles of crude extracts produced by AQP strain cultured under stressed SeaRch conditions (red) vs. non-stressed culture conditions (blue)

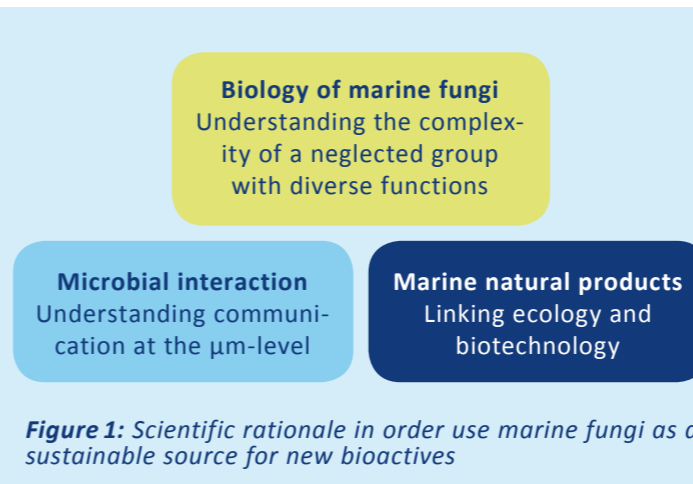
**References**

- <sup>1</sup> Grabowski K., , K.H. Baringhaus and G. Schneider, Natural Product Reports, 2008, 25, 892–904.
- <sup>2</sup> W. Sneader, in Drug discovery; A History ed John Wiley & Sons Ltd., West Sussex, 2005 Ch 1 p. 1; Section 3 Ch 21 p.287.
- <sup>3</sup> T.F. Molinski, D.S. Dalisay, S.L. Lievens and J.P. Saludes, Nature Reviews Drug Discovery, 2009, 8, 69.
- <sup>4</sup> H. J. Field and E. De Clercq, Microbiology Today, 2004, 31, 58.
- <sup>5</sup> J.W. Blunt, B.R. Copp, W.P. Hu, M.H. Munro, P.T. Northcote and M.R. Prinsep, Nat. Prod. Rep., 2009, 26,170.
- <sup>6</sup> Mearns-Spragg, Jukes, K & Yan L. "Microbial secondary metabolites" (2007) GB0701021.8

## Genome based methods for the exploration of natural products from marine fungi for the treatment of cancer

Antje Labes | Kieler Wirkstoff-Zentrum at Helmholtz Center for Ocean Research GEOMAR, Germany

Marine Fungi represent an excellent source for new bioactives<sup>1,2</sup>. However, understanding the complexity of a neglected group with diverse functions is still far away. Marine fungi are not well characterised and underutilised in terms biotechnological application. A comprehensive scientific rationale has to be applied (fig. 1).

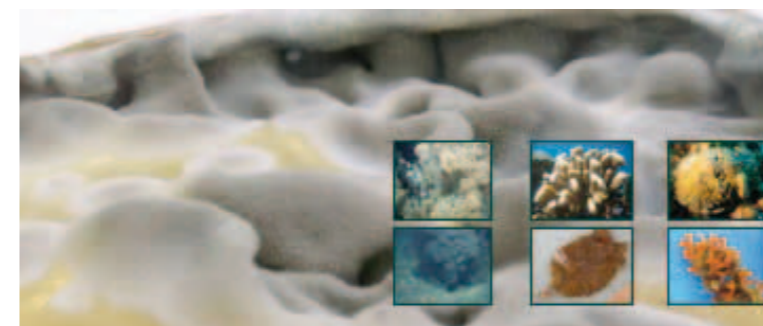


Here, we demonstrate the sustainable exploitation of marine natural resources providing appropriate culture conditions for the group of marine fungi, thus enabling efficient production of marine natural products in the laboratory and also in large scale cultures, avoiding harm to the natural environment. In the focus are new anti-cancer compounds.

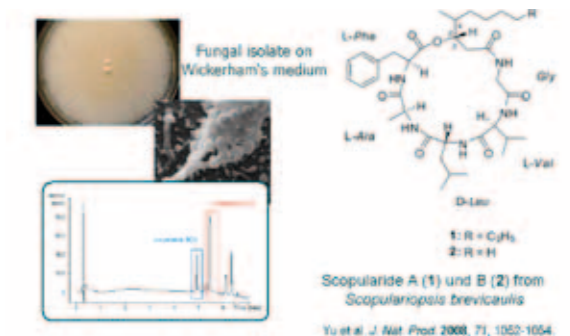
Beside isolation of new fungal strains from unique marine habitats, the molecular development of effective producer strains is in the focus. Genomes of selected candidate strains originating from our unique strain collection of marine fungi are characterised with respect to secondary metabolite production. This knowledge is used to optimise production using molecular methods.

Since in the case of secondary metabolites, genetic potential does not mean production, comprehensive approaches are needed, combining culture based methods as: targeted isolation, screening of strain libraries, *in situ* enrichments as well as culture independent methods as: specific genetic screening, metagenomics, genomics. The KiWiZ holds the EU's largest culture collection of marine fungi (>10,000 strains, fig. 2) – molecular methods can be applied to improve selections of strains for screening.

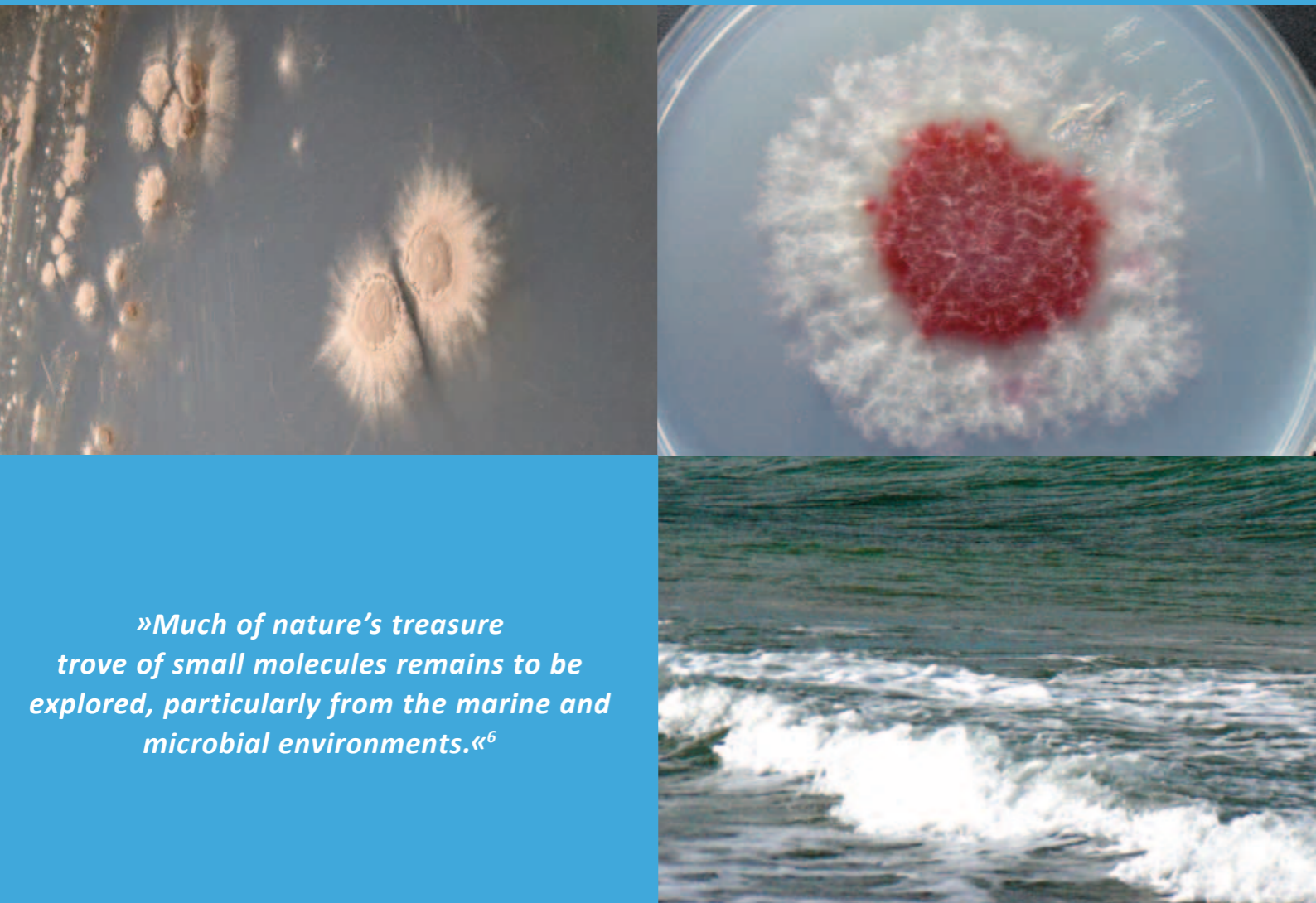
Since in fungi secondary metabolite genes are organized in clusters, the assumption was made that biosynthetic genes are markers for the selection of producers of secondary metabolites. Concerning (marine) fungi, a genetic screening for polyketide synthetase (PKS) genes was available but not for non-ribosomal peptide synthetase (NRPS) genes<sup>3,4</sup>. We were able to verify PKS genes in marine fungi using the Nicholson system (Klotz unpublished). The question was: Are these genes good markers? This was only shown for some groups, eg. *Penicillium*, which is not surprising (Kramer, unpublished) – so genomes are needed. As first candidate, *Scopulariopsis brevicaulis* was chosen (fig. 3.)



**Figure 2:** The Kieler Wirkstoff-Zentrum at GEOMAR holds a culture collection of appr. 10,000 marine fungi and has access to different marine habitats. A particular high percentage of microorganisms associated with various macroorganisms (if compared to free living microbes in ocean waters) are able to produce substances interesting for pharmaceutical production. Therefore sponges, bryozoa, algae, corals and other marine macrobes (small photos) are sampled to gain new strains.



**Figure 3:** *Scopulariopsis brevicaulis* from a marine sponge was chosen for genome sequencing<sup>5</sup>



»Much of nature's treasure trove of small molecules remains to be explored, particularly from the marine and microbial environments.«<sup>6</sup>

Compounds originating from those approaches will be characterised to the stage of *in vivo* proof of concept ready to enter further drug development in order to valorise the results of an EU FP7 project (MARINE FUNGI, 259625). This approach is an outcome of the project "MARINE FUNGI" within the KBBE framework of EU's FP7. We develop a process concept for these

compounds providing the technological basis for a sustainable use of marine microbial products as result of Blue Biotech. Therefore, we will explore the potential of marine fungi as excellent sources for useful new natural compounds along the added-value chain from the marine habitat to the drug candidate and process concept.

**References**

<sup>1</sup> Cragg GM, Grothaus PG, Newman DJ (2009) Impact of natural products on developing new anti-cancer agents. *Chem Rev* 109:3012–3043.

<sup>2</sup> Imhoff JF, Labes A, Wiese J (2011) Bio-mining the microbial treasures of the ocean: new natural products. *Biotechnol Adv* 29:468–482.

<sup>3</sup> Nicholson TP, Rudd BA, Dawson M, Lazarus CM, Simpson TJ, Cox RJ (2001) Design and utility of oligonucleotide gene probes for fungal polyketide synthases. *Chem Biol* 8:157–178.

<sup>4</sup> Schneemann I, Nagel K, Kajahn I, Labes A, Wiese J, Imhoff JF (2010) Comprehensive investigation of marine Actinobacteria associated with the sponge *Halichondria panicea*. *Appl Environ Microbiol* 76:3702–3714.

<sup>5</sup> Yu Z, Lang G, Kajahn I, Schmaljohann R, Imhoff JF (2008) Scopularides A and B, cyclodepsipeptides from a marine sponge-derived fungus, *Scopulariopsis brevicaulis*. *J Nat Prod* 71:1052–1054.

<sup>6</sup> Newman DJ, Cragg GM (2007) Natural products as source of new drugs over the last 25 years. *J Nat Prod* 70:461 f.

**Improving the Baltic Sea environment and economies: Innovative approaches to the sustainable use of marine resources**

The Baltic Sea Region faces enormous challenges including new installations, fishery declines, excessive nutrient input, the effects of climate change as well as demographic change. But novel technologies and growing knowledge also provide opportunities for new uses of marine ecosystems, which can be both commercially appealing and environmentally friendly. Through increased understanding and promotion of innovative and sustainable new uses of the Baltic Sea, SUBMARINER provides the necessary basis for the region to take a proactive approach towards improving the future condition of its marine resources and the economies that depend on them. It does so by focusing its efforts along four lines of activity:

- **Production of a compendium:** describing current and potential future marine uses by developing a comprehensive inventory of innovative sustainable uses, assessing their environmental and socioeconomic impacts, estimating the market opportunities and the availability of necessary technologies, and describing the gaps and obstacles in the legal framework.
- **Development of a roadmap:** recommending necessary policy steps to promote beneficial uses and mitigate against negative impacts, including suggested legal changes (e.g. spatial plans), environmental regulations and/or economic incentives.
- **Implementation of regional development activities:** testing new uses in real conditions, conducting feasibility studies for new uses in specific areas, assessing technological and financial needs, estimating impacts on environmental and socioeconomic conditions, and evaluating specific legal constraints.
- **Building a network:** creating a selfstanding, independent network for sustainable innovative marine uses and stimulating cooperation among relevant players through virtual and real networking, information exchange and cooperation events.



[www.submariner-project.eu](http://www.submariner-project.eu)

**Partners**

- Poland:**
  - **Lead Partner:** The Maritime Institute in Gdańsk
  - Gdańsk Science and Technology Park
- Germany:**
  - Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
  - Norgenta North German Life Science Agency
  - Kieler Wirkstoff-Zentrum am GEOMAR | Helmholtz Centre for Ocean Research Kiel
  - University of Rostock
  - BioCon Valley Mecklenburg-Vorpommern e.V.
- Denmark:**
  - ScanBalt
  - Lolland Energy Holding
- Sweden:**
  - Royal Institute of Technology (KTH)
  - The Royal Swedish Academy of Sciences
  - Trelleborg Municipality
- Estonia:**
  - Tallinn University of Technology
  - Entrepreneurship Development Centre for Biotechnology & Medicine
- Lithuania:**
  - Klaipeda University Coastal Research and Planning Institute
  - Klaipeda Science and Technology Park
- Latvia:**
  - Ministry of Environmental Protection and Regional Development of the Republic of Latvia
  - Environmental Development Association
- Finland:**
  - Finnish Environment Institute – SYKE

