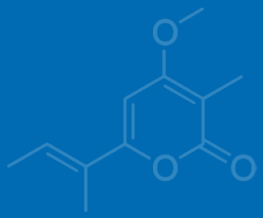
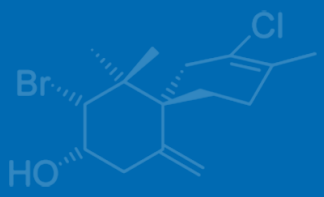
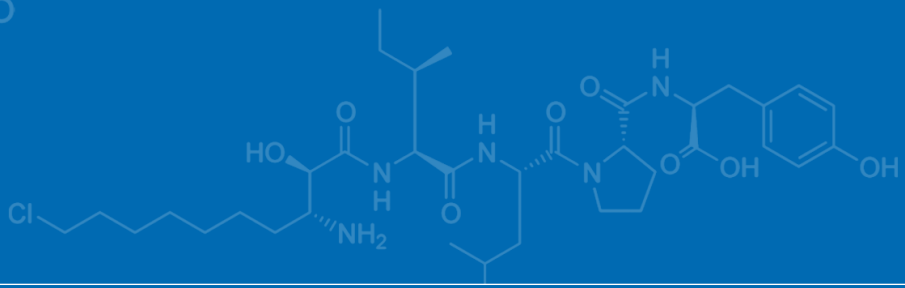
FROM FUNCTIONAL GENOMICS TO NATURAL PRODUCTS OF MARINE MICROORGANISMS

Marine
Functional Genomics
New Drugs
Enzymes
Metagenomics







June 21-24, 2006

Greifswald, Germany
Alfried-Krupp-Wissenschaftskolleg

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Scope of the Conference

During evolution the extreme marine environmental conditions have produced adaptation strategies in marine microorganisms that are different from their terrestrial counterparts. This involves new natural products that are different from known structures of terrestrial organisms. However, the exploration of new drugs, enzymes or biochemical capabilities of marine origin is difficult. Less than 1 % of marine microorganisms can be cultivated so far, and for a negligible number of these known marine microorganisms genetic tools are available. Metagenomics, genome sequencing and the techniques of functional genomics make it possible to visualize potential metabolic and biochemical capabilities of even unculturable marine cells. The conference will present recent results of these new research fields and discuss the potential of molecular methods for the discovery of new natural compounds from marine microorganisms.

Organized by
Ernst-Moritz-Arndt-Universität Greifswald
Institut für Marine Biotechnologie e.V.

Wednesday, June 21, 2006

Thursday, June 22, 2006

Natural products II

Chair: Nobuhiro Fusetani

09:00 – 09:30 Proksch, P., Ebel, R., *Düsseldorf, Germany*
Sponge-derived fungi – a prolific source for new natural products

09:30 – 10:00 Schulz, B., *Braunschweig, Germany*
Secondary metabolites of fungi isolated from algae, sponges and plants from marine habitats

10:00 – 10:20 Kelman, D., Berman-Frank, I., *Bar-Ilan, Israel*
Antioxidant chemical defense by the bloom-forming marine cyanobacterium, Trichodesmium spp.

10:20 – 10:40 Dobretsov, S., *Kiel, Germany,* Qian, P.Y., Dahms, H.-U., *Kowloon, Hong Kong*
Comparison of antifouling activity and microbial diversity of congeneric sponges from different geographic regions

10:40 – 11:00 König, T., Walter C., *Erlangen, Germany*
Screening and biosynthesis of microalgae compounds with activities towards human pathogen viruses

11:00 – 11:30 Coffee break

Chair: Gabriele König

11:30 – 12:00 Hill, R.T., Peraud, O., *Baltimore, USA,* Kasanah, N., *University, USA,* Anderson, M. A., Enticknap, J.J., *Baltimore, USA,* Hamann, M.T., *University, USA*
Microbial sources for pharmaceutically important compounds derived from marine invertebrates

12:00 – 12:20 Hertweck, C., *Jena, Germany*
Biosynthesis of Toxins by Endofungal Bacteria

12:20 – 12:40 Hagemann, M., *Rostock, Germany*
Salt acclimation of cyanobacteria - molecular biology and possible application

12:40 – 13:00 Lukowski, G., *Greifswald, Germany*
Micro particles for controlled release of natural products

13:00 – 14:00 Lunch (sponsored by Bruker)
Brauman, U., *Bruker*
Coupling of LC-MS/NMR and statistical approaches for the analysis of metabolites in complex mixtures

Metagenomics II

Chair: Jörn Piel

14:00 – 14:30 Müller, R., *Saarbrücken, Germany*
From terrestrial genome analysis to marine natural product producers?

14:30 – 15:00 Streit, W., *Hamburg, Germany*
Metagenomes of biofilms: an intriguing resource for the isolation of novel biocatalysts and other bioactive molecules

15:00 – 15:30 Golyshin, P.N., *Braunschweig, Germany,* Ferrer, M., *Madrid, Spain,* Yakimov, M.M., *Messina, Italy,* Timmis, K.N., *Braunschweig, Germany*
New proteins from (meta)genomes of marine hydrocarbon-degrading organisms and their consortia

15:30 – 15:50 Hong, K., *Chengxi, China*
Exploiting bioactive genes from mangrove soil by metagenome approach

15:50 – 16:20 Coffee break

Functional genomics II

Chair: Michael Hecker

16:20 – 16:50 Glöckner, F.O., Bauer, M., Teeling, H., Lombardot, T., Würdemann, C., Wecker, P., Ellrott, A., *Bremen, Germany*
Genome analysis of environmentally relevant marine bacteria – Lessons from the Rhodopirellula baltica SH1T genome

16:50 – 17:10 Bauer, M., Teeling, H., Richter, M., Lombardot, T., Quast, C., Würdemann, C., Simonato, F., Wecker, P., Ellrott, A., Amann, R., Glöckner, F.O., *Bremen, Germany*
Functional genome analysis of the marine bacterium 'Gramella forsetii', a predicted specialist for degradation of polymeric organic matter

Friday, June 23, 2006

End of Symposium

Saturday, June 24, 2006

Excursion to the Island Usedom

Functional genomics I

Chair: Michael Hecker

Exploring unknown Microbial Diversity by environmental genomics

Rudolf Amann*

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Molecular fingerprints indicate the presence of large numbers of so far uncultured microorganisms – potentially millions of species - in common marine habitats such as, e.g., temperate and cold marine waters or sediments. More than 100 years of scientific microbiology have merely yielded an initial insight into this immense microbial diversity. Today, we often know little more of these microbes than the sequence of a single gene, usually the one coding for the 16S rRNA. From comparative sequence analysis affiliation - or lack of affiliation - with cultured bacteria can be deduced.

Fluorescence in situ hybridization with rRNA-targeted oligonucleotide probes yields quantitative data on the abundance and spatio-temporal distribution of microorganisms. The use of horseraddish peroxidase-labeled oligonucleotides is currently increasing the sensitivity of this method considerable. By means of automated microscopy and flow cytometry multiple samples can be analyzed. Thereby abundant marine "key species" have been identified. By targeted isolation more and more representative pure cultures are retrieved. This shows that, although isolation of pure cultures of many marine prokaryotes remains difficult, we need to continue and strengthen our efforts in this respect. Full genome sequencing of many isolates is currently ongoing and this undoubtedly will result in a better understanding of the environmental adaptations and the biotechnological potential. Our institute in Bremen is currently involved in the genome analysis of several cultured marine bacteria via the German REGX project and the EU Network of Excellence "Marine Genomics Europe".

The shot gun sequencing of "metagenomes", a new term defined as the sum of the genomes that can be found in a particular habitat, has started. The huge effort Craig Venter is currently only one of several attempts to analyze the microbial diversity of the world oceans as detailed as possible. Along similar lines, we are retrieving and analyzing large genomic fragments from marine planktonic and benthic communities, e.g., from recently discovered assemblages catalyzing the anaerobic oxidation of methane. In this respect it has to be noted that untargeted approaches will necessary oversample the more abundant taxa whereas the vast majority of true marine microbial diversity will be remain undiscovered.

This is where more targeted approaches including the enrichment and cultivation methods come in. Not only will a meaningful interpretation of the increasing flood of "environmental sequences" require a complete set of strategically selected, coherently annotated, high-quality full genome sequences of pure cultures of marine microbes, we also need functional studies on lab cultures to learn more about the numerous "unknown ORFs". There is, however, no doubt that the next decade of marine genomics will teach us much about the role of marine microbes in the global cycling of elements, and, as a spin-off of basic research also result in the discovery of new natural products.

Functional genomics I
Wednesday, 09:30 – 10:15

Exploring natural product pathways in invertebrate metagenomes

Jörn Piel[1]*, D. Butzke[1], L. Fieseler[2], U. Hentschel[2], G. Wen[3], M. Platzer[3]

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[2] Institute for Molecular Infection Biology, University of Würzburg, Germany
[3] Leibniz Institute for Age Research, Jena, Germany.

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Many marine natural products from marine invertebrates have been suspected to be produced by symbiotic bacteria. However, due to the general inability to cultivate the suspected producers their study remains challenging. We follow a metagenomic approach to gain insights into the chemistry and biology of uncultivated symbionts. Total animal DNA containing all genomes is cloned to generate complex libraries, which are screened for biosynthesis genes of interest. These can then be attributed to the corresponding producer. The feasibility of this approach was first demonstrated by using the beetle Paederus fuscipes as model, the source of the cytotoxic polyketide-nonribosomal peptide pederin. The entire set of pederin genes was isolated and shown to belong to a symbiotic bacterium with close relationship to Pseudomonas aeruginosa, which colonizes the beetle in large numbers[(1)]. Recently a first draft of the symbiont genome was completed and is now being analyzed for factors that determine symbiosis or culturability. The metagenomic strategy is also applied to highly complex metagenomes of marine sponges. From Theonella swinhoei, genes involved in the biosynthesis of the onnamides[(2)] were isolated and found to be of bacterial origin. The talk discusses recent progress in the development of techniques to identify, isolate and express genes from symbiotic sources.

[(1)] J. Piel, Proc. Natl. Acad. Sci. USA 99, 14002-14007 (2002).
[(2)] J. Piel, D. Hui, G. Wen, D. Butzke, M. Platzer, N. Fusetani, S. Matsunaga, Proc. Natl. Acad. Sci. USA 101, 16222-16227 (2004).

Functional genomics I
Wednesday, 10:15 – 10:50

Metagenomics I

Chair: Thomas Schweder

Accessing Metagenomes for industrial applications

Juergen Eck*, Guido Meurer, Renate Schulze, Patrick Lorenz

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Currently there is a global political drive to promote „white biotechnology" as a central feature of the sustainable economic future of industrialised societies. The access to biological resources and subsequent implementation of biotechnological applications promises improvement for existing process or could enable novel product ideas (Lorenz & Zinke, 2005). Historically biotechnology has missed to screen 99% of existing microbial resources. As a consequence strategies of directly cloning and screening of environmental DNA are becoming increasingly popular to circumvent this restriction (Handelsman, 2004; Venter et al., 2004). Comprising the genetic blueprints of entire microbial consortia the metagenomes provide functionally meaningful molecular sequence space in terms of novel enzymes and biocatalysts, the construction of optimised „designer-bugs" and the identification of new natural compounds for industrial applications (Lorenz & Eck, 2005).
Different industries are interested in exploiting the resource of uncultivated micro-organisms:
Ideal biocatalyst - Instead of designing a process to fit a mediocre enzyme, it is conceivable that the uncultivated microbial diversity, together with in vitro evolution technologies, might be used to find a suitable enzyme that that optimally fits process requirements.
Elusive metabolites - Many pharmacologically active secondary metabolites are produced by bacteria that live in complex consortia or by bacteria that inhabit niches that are difficult to reconstitute in isolated production hosts. So, the cloning and heterologous expression of biosynthetic gene clusters that encode secondary metabolites is the most straightforward method of accessing their biosynthetic potential.
Novelty - For industries a single enzyme backbone with superior functionality that has an entirely new sequence or a single pharmacologically active natural compound with a novel structure and new mode of action would be useful to avoid infringing competitors´ intellectual property rights.
Here we report on the evaluation and expression of high molecular weight DNA from metagenome cloned as Large-Insert Libraries (LIL®s) as well as the development of flexible LIL expression systems for heterologous expression of metagenome DNA in biosynthetically competent expression hosts. The biosynthetic potential was examined by screening for the production of novel enzymes as well as novel anti-microbial activities and the presence of secondary metabolite producing biosynthetic gene clusters.

Handelsman J. (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. 68(4):669-85.
Lorenz P. and Eck J. (2005) Metagenomics and industrial applications. Nature Reviews Microbiology 3: 510-516
Lorenz P. and Zinke H. (2005) White biotechnology: differences in US and EU approaches ? Trends Biotechnol 23 (12): 570-574
Venter JC et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304 (5667): 66-74.

Metagenomics I
Wednesday, 11:20 – 11:50

Making Magnets by Microbes: New insights into magnetosome formation in magnetotactic bacteria

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Magnetotactic bacteria are able to navigate along magnetic fields in their aquatic habitats based on specific intracellular structures, the magnetosomes, which are nanometer-sized crystals of magnetite (Fe3O4). Magnetosome formation is an intriguing example for a biologically controlled mineralization process, which has been poorly understood at the molecular level for many years. Unlike magnetite produced in inorganic systems, bacterial magnetosome particles display a variety of species-specific morphologies, have narrow size distributions and highly defined structural and magnetic characteristics. Because of their unique features, bacterial magnetosomes have the potential to yield biogenic magnetic nanoparticles for use in a number of applications, such as in immobilization of bioactive compounds, magnetic separation, and others.

Knowledge about magnetosome biomineralization has vastly increased recently by a combined ecological, biochemical, genetic and genomic approach. Magnetosome biomineralization involves intracellular iron accumulation of more than 4% of the dry weight. Magnetite precipitation occurs within a compartment provided by the magnetosome membrane. Proteomic analysis of the magnetosome membrane in our model organism Magnetospirillum gryphiswaldense revealed 18 magnetosome-specific polypeptides, which are encoded by several mam-operons within a large, highly variable genomic "magnetosome island" (MAI). Functional genomic analysis of the MAI indicated the essential role of mam genes in magnetosome-directed iron transport, magnetosome chain assembly and control of magnetite crystallization. Recent approaches for the genetic characterization of biomineralization include the genomic and metagenomic analysis of diverse cultivated and uncultivated magnetotactic bacteria. Genetic technology is also being used to design and produce functionalized magnetic nanoparticles with tailored characteristics.

Metagenomics I
Wednesday, 11:50 – 12:20

Universal gene cloning and heterologous expression of marine natural product biosynthetic pathways

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Natural products from symbiotic associations between marine invertebrate and microorganisms show exceptional promise as pharmaceuticals in many therapeutic areas. The predicted lack of an economic and sustainable global market supply for production of marine-derived drugs, due to difficulty of synthesis, is often cited as the main obstacle to invest in and exploit these otherwise exciting bioactive compounds. Different strategies have been evoked to overcome this impediment as long-term harvesting of wild stocks from the environment is considered unsound, and other modes of production based on biosynthesis, such as aquaculture, have not yet been proven as reliable. One option is to clone the genes encoding the biosynthetic expression of a lead metabolite into a surrogate host suitable for industrial-scale fermentation. To facilitate this goal we are developing a universal system to clone and express genes responsible for biosynthesis of natural products from both eukaryotic and prokaryotic partners of marine symbioses. The ability to harness the complete meta-transcriptome of entire biosynthetic pathways is particularly valuable where the biogenesis of a target natural product occurring within a complex symbiotic association is unclear.

Metagenomics I
Wednesday, 12:20 – 12:40

Natural products I

Chair: Ulrike Lindequist

Antitumor Leads from Japanese Marine Invertebrates

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Ara-C (cytarabine) is still only a marine-derived anticancer drug, although much efforts toward the development of anticancer drugs from marine organisms have been made since the early 1970s, which actually led to the isolation of a large array of compounds having potent cytotoxicity as well as novel structures. For over 25 years, we have been also involved in the discovery of antitumor leads from Japanese marine invertebrates, especially sponges, by employing cell-based and mechanism-oriented bioassays, which resulted in the isolation of a diverse range of cytotoxic compounds, including terpenoids, polyacetylenes, macrolides, peptides and alkaloids. In the earlier stage of our work, we used "echinoderm embryo assay", which led to the isolation of such important compounds as mycalisines, kabiramides/mycalolides and calyculins. Cytotoxicity tests using tumor cell lines resulted in the discovery of 13-deoxytedanolide, theopederins, polytheonamides, and ritterazines. More recently we employed the assay based on "morphological changes" in rat 3Y1 fibroblasts, and isolated shishijimicins, highly cytotoxic enediynes, from a tunicate. A variety of enzymes are associated with human cancers, and inhibitors of such enzymes are potential antitumor leads. We have isolated a number of interesting compounds that inhibit such enzymes. Azumamides are cyclic peptides inhibiting histone deacetylase isolated from a marine sponge, while dictyodenrines and axinelloside A were isolated as telomerase inhibitors from marine sponges. Interstingly, ageladine A, an inhibitor of matrix metalloprotease isolated from a marine sponge, showed antiangiogenic activity. The structures, biological activities and modes of action of these antitumor leads will be discussed.

Marine and "Marine-like" Natural Products from Microorganisms

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The marine environment is distinguished by unique groups of organisms being the source of a wide array of fascinating structures. The fact that many marine invertebrates contain endo- and epibiotic microorganisms and that invertebrate-derived natural products are structurally related or even identical to bacterial metabolites suggests a microbial origin for some of these compounds. Whereas so far most symbiotic marine microorganisms cannot be isolated and cultured, it is possible to obtain "marine-related" natural products from microorganisms of diverse habitats, including terrestrial and fresh water environments. We focus on the isolation of microbial taxa from diverse habitats, but phylogenetically related to marine-invertebrate symbionts and on microorganisms living associated with marine organisms.

Recently we studied a cyanobacterial strain, which was placed within the order Oscillatoriales, genus Tychonema, based on its 16S rDNA sequence. The structures and absolute stereochemistry of three cyclic peptides, brunsvicamides A, B and C were identified. Most unusual and rare is the N-methyl-N´-formylkynurenin moiety found in brunsvicamide C. Brunsvicamide A and B, however are close to identical to the mozamides, which were formerly isolated from a sponge belonging to the family Theonellidae. Brunsvicamides are selective inhibitors of a Mycobacterium tuberculosis associated phosphatase.

Bacteria of the genus Herpetosiphon belong to the phylum Chloroflexi. They are gram negative gliding bacteria with an unusual cell wall composition. Herpetosiphon spp. have proboscislike protuberances that resemble myxobacterial fruiting bodies, they are however phylogenetically very different from the latter. From a halotolerant Herpetosiphon sp. we isolated siphonazole. It is a remarkable natural product, composed of a styrene residue, two oxazole rings connected via a C2-bridge, and a very unusual, amide bonded diene containing side chain. Even though partial structural motifs such as the oxazole rings of this compound resemble those of myxobacterial and marine natural products, the overall composition makes this compound the first member of a new structural class.

Ascochyta salicorniae is a marine-derived fungus isolated as an endophyte from a North Sea alga. This fungus is distinguished by the extraordinary diversity of its polyketide metabolism giving rise to unprecedented compounds with a spiro-ketal substructure and new carbon skeletons.

All of these microorganisms are relatively easy to cultivate, which ensures a secure supply of compounds. Culturable microorganisms also allow the elucidation of biosynthesis using labeled precursors, and finally the cloning and modification of the biosynthetic gene clusters.

Natural products I
Wednesday, 15:00 – 15:30

Drug discovery of marine microorganisms as source of new antitumor compounds at PharmaMar

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The marine environment is a virtually untapped source of novel micro-biodiversity and therefore of new metabolites. PharmaMar, a spanish biotechnology company, devoted to the research and development of marine natural products for the treatment of human cancers, is developing a drug discovery program using marine samples (invertebrates and microorganisms). The microorganism drug discovery activities involve the isolation, molecular dereplication, culture, screening, molecular taxonomy, scale-up and purification of new antitumor metabolites. To date, some promising "preclinical candidates" have been isolated such thiocoraline, IB1211, and other NCE's. In conclusion, the marine microbial diversity potencially represents an extraordinary abundant source of chemical diversity for industrial application.

Screening for Cytotoxic Compounds from Marine Bacteria

Els W Maas[1]*, Vicky L Webb[1], Anna Y Kilimnik[1], Debbie A Hulston[1], Silke Hopf[1,2], Simon Hinkley[2], Sarah Reader[2], Andrea Thomson[2]

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Marine bacteria are a proven potential source of novel compounds for the pharmaceutical industry. About 20 000 secondary metabolites have been described from microbes with many different properties, ranging from colour pigments to antibacterial compounds. Cytotoxic compounds of bacterial origin have been discovered over the years and continue to be described.

In this work, New Zealand marine bacteria were isolated from a variety of sources using solid media. The isolates were purified, characterised and broadly classed into taxonomic groups. The isolates were initially screened through both a novel co-culturing screening system developed in our laboratory followed by a cell based MTT cytotoxic assay, using the human fibroblast cell line MRC-5. For the MTT assay all the isolates were fermented in a seawater based media with the bacterial supernatants screened in the MTT assay.

Three hundred and thirty isolates were obtained of which 90 were presumptively identified as marine actinomycetes. Seven percent of the isolates were positive in our co-culture screening assay. Of these 2.5% were also positive in the MTT assay whilst a further 3% showed activity only in the MTT assay. The isolates that were positive in any one of the initial screening assays were re-fermented in a different medium and extracted using solvents. These extracts were re-screened in the MTT assay against two different cell lines. To date, two isolates have shown specific activity against the epithelial lung carcinoma cell line A549 compared to a human non-cancer fibroblast cell line MRC-5. Chemical structure elucidation of these two isolates and other positive isolates is ongoing.

Our results to date show that the simple co-culture screen is able to identify cytotoxic compounds produced by marine bacteria. However, it appears that the MTT assay and our co-culture screen do not appear to be measuring the same activity as some isolates were only positive in one or the other assay. Further work will have to be conducted on establishing what the two different screens are measuring and specifically; the nature of the cytotoxic principles.

Natural products I
Wednesday, 16:00 – 16:20

Symbiosis I

Chair: Rolf Müller

Porifera a reference phylum for evolution and bioprospecting: Or – the power of marine genomics

Werner E.G. Müller[1]*, H. Ushijima[2], S.I. Belikov[3], I.M. Müller and H.C. Schröder

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[2] Department of Developmental Medical Sciences, Institute of International Health, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
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The term Urmetazoa, as the hypothetical metazoan ancestor, was introduced to highlight the finding that all metazoan phyla including the Porifera [sponges] derived from one common ancestor. Analyses of sponge genomes, from Demospongiae, Calcarea and Hexactinellida have permitted the reconstruction of the evolutionary trail from Fungi to Metazoa. This has provided evidence that the characteristic evolutionary novelties of Metazoa existing in Porifera share high sequence similarities and in some aspects also functional similarities to related polypeptides found in other metazoan phyla. It is surprising that the genome of Porifera is large and comprises more genes than Protostomia and Deuterostomia. On the basis of solid taxonomy and ecological data, the high value of this phylum for human application becomes obvious especially with regard to the field of chemical ecology and the hope to find novel potential drugs for clinical use. In addition, the benefit of efforts in understanding molecular biodiversity with focus on sponges can be seen in the fact that these animals as "living fossils" allow to stethoscope into the past of our globe especially with respect to the evolution of Metazoa.
The value of some selected secondary metabolites, all obtained from sponges and their associated microorganisms, is highlighted exemplarily. Examples of compounds, already in medical use, or being considered as lead structures, or as prototypes for the interference with metabolic pathways common from sponges up to human, are discussed. It is outlined that the skeletal elements, the spicules, can serve as blueprints for new biomaterials, especially biosilica, which might be applied in biomedicine.
Such compounds and biomaterials have been isolated/studied by members of the German Center of Excellence BIOTECmarin as well as the EU MARIE CURIE RESEARCH TRAINING NETWORK (MCRTN) "BIOCAPITAL".

W.E.G. Müller et al. Intern. Review of Cytology 235, 53-92 (2004).
W.E.G. Müller et al. Naturwissenschaften 90, 103-120 (2003).
W.E.G. Müller et al. Marine Biotechnol. 6, 105-117 (2004).
W.E.G. Müller et al. FEBS Journal 272, 3838-3852 (2005).
W.E.G. Müller et al. J. Struct. Biol. 153, 31-41 (2006).
M. Wiens et al. J. Biol. Chem. 280, 27949-27959 (2005).
H.C. Schröder et al. J. Biol. Chem.; in press.

Characterizing microbial diversity and function of marine-sponge associated microbial consortia

Ute Hentschel[1]*, Jörn Piel[2], Lubomir Grozdanov[1], Matthias Scheuermayer[1]

[1] Research Center for Infectious Diseases, University of Würzburg, Germany, Röntgenring 11, D-97070 Würzburg, Germany
[2] Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Germany, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany

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Marine sponges (Porifera) are a rich source of secondary metabolites. Because many species are associated with enormous amounts of microorganisms contributing up to 40 - 60% of the sponge biomass they can be considered as 'microbial fermenters' that hold a largely untapped potential for biotechnological applications(1). The implementation of cultivation-independent techniques using the 16S rRNA gene as a phylogenetic marker has provided unprecedented insights into the microbial community composition of these animals. Representatives of at least eight different phyla, many of which contain few or no cultivated representatives, have been identified as specific members of the sponge-associated microbiota. Moreover, a novel candidate phylum, termed 'Poribacteria', was recently described that is characterized by cell compartimentation in form of a nucleoid-like structure(2). Because the 'Poribacteria' have not been cultivated, a metagenomic approach was used to gain insights into their genomic properties and possibly physiological/functional features(3). Metagenomic libraries also serve as resources for the identification, expression and characterization of enzymes and secondary metabolite biosynthetic operons of biotechnological relevance. An overview over the recent insights into the microbial diversity and possible functions of marine sponge-associated microbiota will be presented.

(1) Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microb Ecol 55(2):167-177.
(2) Fieseler L, Horn M, Wagner M, Hentschel U (2004) Discovery of a novel candidate phylum 'Poribacteria' in marine sponges. Appl Environ Microbiol 70(6): 3724-3732
(3) Fieseler L, Quaiser A, Schleper C, Hentschel U (2006) Analysis of the first genome fragment from the marine sponge-associated, novel candidate phylum 'Poribacteria' by environmental genomics. Environ Microbiol 8(4):612-624.

Symbiosis I
Wednesday, 17:20 – 17:50

Riftia pachyptila: The poster child for symbiosis with chemoautotrophic bacteria

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*e-mail: hfelbeck@ucsd.edu

The vestimentiferan tubeworm Riftia pachyptila lives in the vicinity of deep sea hydrothermal vents and is characterized by its symbiosis with chemoautotrophic bacteria. Even though this kind of symbiosis has been found in many other organisms after the initial discovery in Riftia, this deep-sea organism is still the best investigated example. The animal and its biotope will be introduced.
The metagenome of the symbionts of the bacterial symbionts of the tubeworm has been analyzed and mostly annotated. The results of biochemical and physiological experiments will be compared with predictions based on genomic sequences.

Symbiosis I
Wednesday, 17:50 – 18:10

Functional genome analysis of the bacterial endosymbiont from the deep sea tube worm Riftia pachyptila

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[2] MPI for Infection Biology, Berlin, Germany
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The vestimentiferan tube worm Riftia pachyptila inhabits deep sea hydrothermal vent areas along submarine ridges. It has established a highly specific symbiosis with sulfide oxidising chemoautotrophic bacteria: R. pachyptila is completely dependent on its bacterial endosymbionts and lacks a digestive system entirely. In a specialised organ, called the trophosome, the endosymbiotic bacteria are supplied with CO_2, H_2S, O_2 and NO_3 – via the circular blood system of their host. They produce organic carbon from CO_2 by means of the Calvin-Benson-Cycle, which is fuelled by the oxidation of reduced sulfur compounds. The extraordinarily high efficiency of this symbiosis makes R. pachyptila one of the fastest growing marine invertebrates.

The genome of the yet uncultured endosymbiont of R. pachyptila has recently been sequenced, the annotation is in progress. Based on the genome sequence physiological aspects of the symbionts can now be analysed on the proteomic level even though experimental data is not accessible.

In our studies we examined the intracellular and the membrane proteome of the bacterial symbionts by two and one dimensional gel electrophoresis (1D and 2D-PAGE). Protein reference maps were established, on the basis of which fundamental metabolic pathways of the chemoautotrophic bacteria were postulated. Among them are the energy generating sulfide oxidation pathway and the reverse TCA cycle. Protein identification was performed using MALDI-ToF mass spectrometry. We also analysed and compared varying symbiotic protein patterns from sulfide rich and sulfide depleted environments using a dual channel imaging software (Delta 2D). Furthermore, we investigated the cellular response of R. pachyptila symbionts to oxidative stress caused by H_2O_2 on the protein level. Our findings result in a detailed and comprehensive physiological picture of the Riftia pachyptila endosymbionts' metabolism, emphasising the immense potential of proteomic methods in the investigation of uncultured and remote microorganisms.

Symbiosis I
Wednesday, 18:10 – 18:30

Pharmacological Screening of Riftia pachyptila

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Marine organisms are an interessting source of biologically active natural products. Especially the broad taxonomic variety of organisms determines a wide chemotaxonomic diversity within marine species. Many secondary metabolites with so far undescribed structural features were isolated in the past. Most of these active compounds are showing cytotoxic, antibacterial or antifungal activities. One of the main strategies for the identification and isolation of new secondary metabolites is the "bioactivity-guided isolation process". This comprehends to the identification of the active principle of an extract attained from the biomass of marine organisms or of the growth medium of the species.

One of the most promising sources of new secondary metabolites are organisms living in extreme habitates like Riftia pachyptila. Riftia pachyptila is a tube worm living in sulfide rich enviroments near hydrothermal vents for instance at depths of 2000-3000m on the East Pacific Rise. R. pachyptila lives in close symbiosis with chemosynthetic bacteria which are located in the trophosome of the tubeworm.

We investigated extracts of the trophosome of R. pachyptila towards their activity against pathogenic bacteria and their cytotoxic activity. Bioactivity guided fractioning and purification directed to compounds which were characterised by HPLC-MS and NMR investigations. Results of the pharmacological screening and the structural characterisation of metabolites from Riftia pachyptila will be presented.

Symbiosis I
Wednesday, 18:30 – 18:50

Natural products II

Chair: Nobuhiro Fusetani

Sponge-Derived Fungi – a Prolific Source for New Natural Products

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Marine natural products continue to draw attention from researchers in academia and industry alike due to their structural uniqueness and their pronounced biological activities. So far over 10,000 different natural products have been isolated mostly from marine invertebrates such as sponges, tunicates, molluscs and others. In recent years the focus of marine natural products chemistry is shifting more and more towards microorganisms which are also prolific sources of interesting new metabolites but in sharp contradiction to most marine macroorganisms can be cultivated in vitro through biotechnological means. Problems in supply have long hampered the progress of marine pharmacology. It is hoped that by increased investigation of marine microorganisms the supply problem can be overcome.

From the various microorganisms that inhabit the sea those that live in association with marine invertebrates (e.g. sponges) or with marine algae appear to particularly interesting with regard to their natural products. Besides bacteria fungi have attracted considerable attention in recent years. Especially sponges have been shown to harbor fungi even though the true nature of this association is not understood at present. As most fungal isolates that have been obtained from marine sponges so far belong to genera well known from the terrestrial environment (e.g. Aspergillus, Cladosporium, Penicillium and others) (Bugni and Ireland, 2004) it appears possible that they originate from the land and are washed into the sea where they are trapped by filter-feeding invertebrates.

Regardless of their true origin sponge-derived fungi have been shown to be prolific sources of new bioactive constituents hitherto unknown from the terrestrial environment. This presentation will focus on the structural diversity and biological activities of natural products recently isolated from sponge-derived fungi by our group (e.g. Brauers et al., 2000; Jadulco et al., 2001; Lin et al., 2003; Hiort et al., 2004).

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Natural products II
Thursday, 9:00 – 9:30

Secondary metabolites of fungi isolated from algae, sponges and plants from marine habitats

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Fungi were isolated from algae, sponges and as endophytes of plants from the shores and waters of various temperate and subtropical locations: North and Baltic Seas, Atlantic Ocean, Mediterranean Sea, Gulf of Mexico. Primarily non-ubiquitous taxa were taken into culture, since previous work had shown that a high proportion of redundant structures is obtained from ubiquitous taxa. As in previous studies, no novel metabolites were found to be produced by ubiquitous genera.

Although the addition of marine salts to the culture medium often improved the growth of the isolates, in general it did not improve secondary metabolite synthesis. Metabolite production was best on solid and solid-substrate media. The structures of 295 different metabolites were elucidated from the culture extracts of 72 fungi; they belonged to diverse structural groups and were products of polyketide, terpenoid, and peptide biosynthetic pathways. Almost all (94%) of these metabolites were active in tests for algicidal, antibacterial, herbicidal and/or fungicidal activities. The proportion of novel metabolites was highest in culture extracts of endophytes from terrestrial marine plants (45%), followed by 36% from fungi associated with algae and only 18% from those isolated from sponges. Since the latter were primarily ubiquitous taxa and thus produced many known metabolites, we hypothesize that these had been filtered. The most novel metabolites were from the genera Phomopsis > Microsphaeropsis > Geniculosporium > Nodulisporium > Coniothyrium.

With respect to biological activity, it was irrelevant from which plant organ the fungi had been isolated. However, it was not irrelevant from which biotope the fungi had been isolated: the highest proportion of biologically active culture extracts was from endophytic fungi from Gomera.

Molecular identification of the fungi associated with Fucus serratus demonstrated that the fungi isolated are not necessarily the most common colonizers of the host. Those isolated with conventional methods were primarily ubiquitous taxa; only 5 of 33 isolates were marine taxa. In contrast, of those identified with molecular methods (those with a higher colonization density), 7 of the 12 taxa are known to be marine fungi. And, having identified these fungi, most could be subsequently isolated. These results demonstrate the importance of using molecular methods to identify the fungi actually associated with marine hosts, since they should be the ones most likely to produce novel metabolites.

In conclusion, fungi from marine habitats are an excellent source of novel and biologically active secondary metabolites.

Natural products II
Thursday, 9:30 – 10:00

Antioxidant Chemical Defense by the Bloom-Forming Marine Cyanobacterium, Trichodesmium spp.

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A fundamental aspect of aerobic metabolism is the harmful generation of partially reduced species of molecular oxygen, generally referred as reactive oxygen species (ROS). The predominant ROS, such as superoxide anion (O_2-) and hydrogen peroxide (H_2O_2), are strong oxidants, leading to protein degradation, enzyme inactivation, lipid peroxidation, and DNA damage. A variety of antioxidants such as the proteins glutathione, superoxide dismutase (SOD), catalase and peroxidase are produced within cells to prevent the deleterious effects of potentially harmful ROS. While the role of antioxidant proteins is well known, the contribution of low-molecular weight bioactive natural products in antioxidant defense is still unclear. ROS are especially common in aquatic ecosystems, where high concentrations are observed in high light surface waters and are additionally produced by photosynthetic, oxygen-evolving, microorganisms. Photosynthetic organisms, especially nitrogen-fixers that must protect the oxygen-sensitive nitrogenase, thus require mechanisms to prevent the deleterious effects of ROS. Marine cyanobacteria of the genus Trichodesmium spp. form extensive blooms in the oligotrophic subtropical and tropical oceans, where they contribute significantly to global nitrogen fixation. In the current study, we discovered potent antioxidant activity produced by Trichodesmium sp. IMS101. The majority of the activity was found to be due to low-molecular weight non-polar natural products, as opposed to antioxidant proteins. The dynamics of the antioxidant production during the growth cycle and in simulated blooms under changing environmental parameters such as pH, temperature and nutrients will be discussed.

Natural products II
Thursday, 10:00 – 10:20

Comparison of antifouling activity and microbial diversity of congeneric sponges from different geographic regions

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Microbial communities of the sponges Callyspongia sp. from Hong Kong and Callyspongia plicifera (Porifera: Demospongia) from the Bahamas were compared with each other and with those from reference substrata using a terminal restriction fragment polymorphism analysis (T-RFLP). The least number of bacterial ribotypes and bacterial isolates were retrieved from Bahamas reference and sponge surfaces, while the bacterial communities from Hong Kong Callyspongia sp. and reference surfaces were more diverse. Microbial communities from the two sponges were different from each other and from reference substrata. GC-MS analysis of sponge dichloromethane extracts revealed that about 50% of the compounds were similar in the two species Callyspongia sp. and C. plicifera, while different from the extracts of Halicondria spp. from both locations. At tissue level (TL) concentrations extracts of Callyspongia spp. predominantly inhibited the growth of the bacterial isolates from reference substrata. Multifactor ANOVA revealed that the source of bacteria (sponge surface, interior, or reference substrata), the geographic location of isolates (Hong Kong or the Bahamas), the sponge extract (from Callyspongia sp. or from C. plicifera), and combinations of these factors gave significant effects in the disc diffusion assay experiments. Both sponge extracts at TL concentration and ten times diluted were toxic to larvae of the polychaete Hydroides elegans and the barnacle Amphibalanus (Balanus) amphitrite. Our results suggest that the two congeneric sponges Callyspongia spp. from different biogeographic regions have different bacterial associates, while producing relatively similar secondary metabolites.

Natural products II
Thursday, 10:20 – 10:40

Screening and biosynthesis of microalgae compounds with activities towards human pathogen viruses

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Microalgae-derived compounds exhibit a novel source of antiviral drug candidates. With regard to drug development a sustainable and reliable supply of natural compounds as pharmaceutical candidates has to be ensured. The use of bioactive compounds from microalgae was not possible previously, mainly due to problems combined with production, isolation and purification of sufficient amount of drug candidates in a reproducible manner. Within this study we show a basic process sequence for the development of appropriate antiviral drug candidates using microalgae extracts.
Target-orientated compounds with potential activities against ß-herpes viruses were isolated from cultures being grown monoseptically in thermal sterilizable photobioreactors of the type „Medusa". Extracts have been generated from biomass and culture supernatant. Antiviral activities were studied against several human pathogen viruses. Characterisation of active compounds has been carried out by chromatography and mass spectrometry methods.
Strong inhibitions of viral replication in the absence of cytotoxic effects were identified for intra- and extracellular extracts from Arthrospira platensis against the human pathogen viruses HCMV, HHV-6A, HSV-1, HIV-1 and vaccinia virus (VV). Additionally, exopolysaccharides (EPS) gathered from Porphyridium purpureum revealed an inhibitory potential against HCMV, HHV-6A and VV.
Investigations about the mode of action indicate an inhibition of an early step of virus infection. In the case of extracellular compounds from A. platensis a complex mode of action against HCMV is postulated. Further on, viral entry might not be the primary target in the inhibition of HIV-1. The isolated compounds or compound derivates might lead to novel drug candidates with a pronounced novel mode of action which might delay resistance formation.

Natural products II
Thursday, 10:40 – 11:00

Microbial sources for pharmaceutically important compounds derived from marine invertebrates

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The supply problem limits the successful development of novel compounds derived from marine invertebrates. Many of these compounds are likely produced by symbiotic microbes rather than by the invertebrates themselves. In some cases, isolation of the "producer" bacteria can ensure economic, sustainable production of important compounds. In two case studies, we have isolated bacteria that produce manzamines and kahalalides. Micromonospora sp. strain M42 from the Indonesian sponge Acanthostrongylophora produces manzamine A, a bioactive compound first found in sponges. Mutagenesis techniques were used to obtain mutants that produce elevated concentrations of manzamine A and one additional manzamine compound. A symbiotic Vibrio sp. that produces kahalalide F was isolated from the mollusk Elysia rufescens. In both cases, sustainable production of the compounds is now possible using fermentation systems. A combination of molecular community analysis and rational selection of culturing conditions may be effective in isolating additional bacteria that produce bioactive compounds from marine invertebrates.

Natural products II
Thursday, 11:30 – 12:00

Biosynthesis of Toxins by Endofungal Bacteria

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Pathogenic fungi generally exert their destructive effects through pathogenicity factors. An important example is the macrocyclic polyketide rhizoxin, the causative agent of rice seedling blight, from the fungus Rhizopus microsporus. The plant disease is typically initiated by an abnormal swelling of the seedling roots caused by rhizoxin without any sign of infection by the pathogen. The phytotoxin exerts its destructive effect by binding to rice ß-tubulin, which results in inhibition of mitosis and cell cycle arrest. Owing to its remarkably strong antimitotic activity in most eukaryotic cells, including various human cancer cell lines, rhizoxin has attracted considerable interest as a potential antitumour drug.
By a series of experiments we could unequivocally demonstrate that rhizoxin is not biosynthesized by the fungus itself, but by endosymbiotic bacteria of the genus Burkholderia. Our unexpected findings unveil a remarkably complex symbiotic-pathogenic alliance that extends the fungus-plant interaction to a third bacterial key player. In addition to unveiling the first fungal-bacterial symbiosis with a clear metabolic function, we were able to produce antitumoral rhizoxin derivatives by a large-scale fermentation of the cultured endosymbiont.

Natural products II
Thursday, 12:00 – 12:20

Salt acclimation of Cyanobacteria - Molecular Biology and Possible Application

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Acclimation to high salt in cyanobacterial cells is based on the active extrusion of toxic ions and the synthesis of compatible solutes. Depending on the type of accumulated compatible solute such as sucrose, trehalose, glucosylglycerol or glycine betaine different salt tolerance levels are realized[1]. Using cyanobacterial model strains (Synechocystis sp. PCC 6803, Synechococcus sp. PCC 7002, Synechococcus sp. PCC 6301, Anabaena sp. PCC 7120) the biochemistry of compatible solute biosynthesis has been elucidated. Based on this knowledge, related genes were searched in the completed genomes of cyanobacterial strains originating from diverse habitats and other prokaryotes. Many genes probably involved in the synthesis of sucrose, trehalose and glucosylglycerol were found. Surprisingly, also genes capable to synthesize glucosylglycerate and related compounds characteristic for Archaea are present in cyanobacterial genomes. Such genes can be used for overexpression in selected host organisms. The transfer of compatible solute synthesizing enzymes into low halotolerant organisms allows two possible applications. First, the salt tolerance of the organism may be improved which is of high importance for crop plants. Second, the compatible solute can be produced in high amounts and may be subsequently used in cosmetics and pharmaceutical industries.

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Natural products II
Thursday, 12:20 – 12:40

Micro particles for controlled release of natural products

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Marine organisms are a prolific resource for new drugs and cosmetics. The effective use of such materials and compounds depends considerable on a suitable application form. Microparticles based on marine organism were produced using a new encapsulation technique.
The microparticles show unique physical properties, which enable them to offer new possibilities for application of biologically active marine compounds. The pure biomass particles had a particles size distribution between 500 nm - 5 µm. Therefore the developed technique under modified conditions is suitable for the production of biomass nano- and microparticles. The small particle size and the increased surface of the biomass and of the marine drugs in nanoparticles should improve their properties and the release behaviour.
Antibacterial and antiviral properties of biomass were significantly increased due to microencapsulating of the marine organisms and the positive properties of all ingredients like proteins, minerals, vitamins, and polyunsaturated fatty acids like α-linolenic acid, arachidonic acid, and eicosapentaenic acid could be maintained. The microparticles decrease skin provoking and restrain the inflammation. The investigations show a synergism of incorporation of drugs in the biomass particles and positive effects of the biomass itself.
Furthermore the particles improve the antibacterial effect of incorporated drugs. As an example, the active substance norlichexanthon, isolated from the marine fungus Humicola fuscoatra Traaen, exhibits no antibacterial effect against MRSA strains in the agar diffusion model, probably because of its low water solubility. However the microencapsulation of the drug in marine biomass particles results in strong antibacterial effects.
Another example are the encapsulated cyanobacterial strains Bio 29 and Bio 33 isolated in the Baltic Sea. Microparticles consisting only of the biomass of these microalgae prevent completely a colonisation of multiresistant Staphylococcus aureus (Hospital germs) on the skin. The microencapsulation is essential to reach this effect. The effect can not be seen with microparticles of commercial available microalgae like Chlorella or Spirulina. In contrast to the usual application of biocide substances the physiological skin flora is maintained. This selective effect offers new possibilities in the fight against the infectious hospitalism.

Natural products II
Thursday, 12:40 – 13:00

Metagenomics II

Chair: Jörn Piel

From terrestrial genome analysis to marine natural product producers?

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Marine natural products often show significant similarity to their counterparts identified from terrestrial microorganisms [1, 2]. One example for such similarity is the jaspamide/chondramide pair of natural products found in jaspis sponges and terrestrial myxobacteria, respectively. Functional assignment of the producing organism of the marine secondary metabolite can be regarded as the first step towards improved production but the real producer organism remains unidentified in most cases. The work presented aims at the identification of the biosynthetic genes for both metabolites based on the finding that most secondary metabolites of microbial origin are biosynthesized by multifunctional megasynthetases. Genomic analysis reveals the presense of numerous polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) in the genomes of well known natural product producers such as actinomycetes and myxobacteria[2]. E.g., the genome of Myxococcus xanthus DK1622 harbours 18 biosynthetic gene clusters but the strain was not known to produce any secondary metabolite until recently. We have shown that PKS and NRPS genes are involved in the formation of myxochelins[3], myxovirescins[4], myxochromides[5] and DKxanthenes[6], the latter being compounds required for spore germination during development.
In the work presented, we have begun to address the question of the origin of the jaspamide/chondramide natural product pair by identifying the chondramid biosynthetic gene cluster from the genome of Chondromyces crocatus Cm c5[7]. A biochemical characterization of the gene cluster will be presented. These results set the stage for a subsequent analysis of the metagenome of the jaspis sponge.

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Metagenomes of biofilms: an intriguing resource for the isolation of novel biocatalysts and other bioactive molecules

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The ability of prokaryotes to adapt, thrive and populate every environment, from hydrothermal vents on the ocean floor to acid mine drainage sites, is related to their metabolic and physiologic diversity. The metagenomic approach offers the possibility not only to analyze the phylogenetic diversity of environmental biofilms, but also to locate genes and operons encoding properties of biotechnological interest[1-3]. Until today metagenomics has led to the discovery and characterization of a wide range of biocatalysts, revealing much about the natural diversity of enzymes and factors, which influence their functions, and making detection and optimization of biocatalysts for specific processes a real possibility. In my laboratory, we have isolated a significant number of novel biocatalysts from metagenomes of microbial consortia and naturally occurring biofilms. Among the biocatalysts identified were amylases, esterases and cellulases. Many of these novel and metagenome derived enzymes have unusual properties and highly interesting substrate specificities.

During my presentation, I will report on the biochemical properties of esterases isolated from the metagenomes of an aquatic biofilm and a contaminated soil. Both genes were overexpressed in E. coli and the recombinant proteins purified. The enzymes are remarkable in the sense that they either have unusual substrate specificities or reveal structural details that are different from known enzymes. In the second part of my presentation I will give an overview on clones isolated from a soil metagenome that are involved in quenching quorum sensing (QS) signals exuded by gram-negative bacteria. Quorum sensing and the production of homoserine lactones are thought to be essential for the growth of many pathogenic biofilms. Therefore, we have initiated work to isolate metagenome clones that degrade homoserine lactones. I will summarize our efforts on the screening strategies employed to identify HSL-degrading clones, molecular properties of selected clones and their effects on P. aeruginosa biofilm formation.

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Metagenomics II
Thursday, 14:30 – 15:00

New proteins from (meta)genomes of marine hydrocarbon-degrading organisms and their consortia

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Recently, a group of so-called hydrocarbonoclastic marine bacteria has been discovered. These bacteria are ubiquitous, possess a narrow substrate profile and have preference for aliphatic hydrocarbons and their derivatives. The genome of one of them, Alcanivorax borkumensis, has recently been sequenced and annotated. Its functional genome analysis revealed metabolic features of the "hydrocarbonoclastic" lifestyle: a large repertoir of monooxygenases and systems for scavenging oligominerals. From its genome a number of novel, not predicted in silico, carboxylesterases with a great enantioselectivity in the kinetic resolution of variety of chiral synthons, were retrieved and characterised.
Oleispira antarctica is another new gamma-proteobacterium that efficiently degrades oil hydrocarbons at low temperatures. Activity-based screening of its genome library revealed a new carboxylesterase that was poorly expressed in E. coli. Trying to co-express this enzyme with Cpn60 chaperonin from Oleispira in E. coli to facilitate better enzyme solubility, we have discovered that E. coli became capable of growth at temperatures as low as 0°C. The consequent study of this phenomenon revealed a small group of essential proteins (Dps, ClpB, RpsB and DnaK) whose cold denaturation causes the systems failure in E. coli at low temperatures.
We performed an activity-based study of a metagenome library derived from the oil-degrading marine community from a brine-seawater chemocline of Urania deep Mediterranean hypersaline anoxic basin. Among few distinct groups of carboxylesterases retrieved from the librariy some enzymes exhibited unusual, habitat-specific characteristics (preference for high hydrostatic pressure, anoxia and high salinity). One exhibited an unusual structural signature incorporating three catalytic active centers mediating distinct hydrolytic activities and an adaptive tertiary-quaternary structure that altered between three molecular states, depending on the prevailing physicochemical conditions. Some of the esterases had high activities, specificities, enantioselectivities, and exceptional stability in polar solvents, and exhibited a good potential for industrial biotransformations.

Metagenomics II
Thursday, 15:00 – 15:30

Exploiting bioactive genes from mangrove soil by metagenome approach

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In order to exploit the most of microbial diversity resources to find novel bioactive compounds or genes, mangrove soil metagenome libraries were constructed. The soil employed in this project was determined based on the bioactivity survey of mangrove marine organism on culture dependant level with samples from different mangrove locations and different mangrove species in tropical and subtropical area in China, and 16S rDNA biodiversity analysis on culture independent level as well. DNA were directly extracted from the selected mangrove soil, fragments of the soil DNA were ligated to an expression plasmid pBluescript SK+, transformed to surrogate host Escherichia coli DH 5?, and the white clones out on LB medium were selected to accumulate libraries. More than 7000 of positive clones with average insert size of 2.7kb-5.5kb were collected. The liberary was screened for bioactivity against Staphylococcus aureus, Candida abbicans and tumor cell B16, employing a high though put screening method of methylene blue (MB) method and MTT. Novel amylase was also screened among this library. Three clonies (4520?4127?4543)with anti-bacteria activity were found out from this library by methylene blue method. These three colonies were sequenced, after blasting in GenBank, among most-similar sequences in responded to clone 4520 and Clone 4127 sequence, it is only 54% and 58% similarity, respectively. It is suggested that they may be new sequences. As to clone 4543, 98% similarity of the known sequences was resulted after blasting.

Functional genomics II

Chair: Michael Hecker

Genome Analysis of Environmentally Relevant Marine Bacteria – Lessons from the Rhodopirellula baltica SH1T Genome

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The technological power of high-throughput sequencing and functional genomics has revolutionized our capabilities to examine the genetic complexity of organisms at the whole genome level. Within the last years, more than 300 microbial genomes have been successfully sequenced and nearly 1000 are currently in progress. Nevertheless, a closer look at the origin of these organisms reveals that the majority of them are of medical or biotechnological interest and environmentally important organisms have only rarely been targeted so far. Since the oceans cover 70% of the Earth's surface and contain an extraordinary diversity of life, the Department of Molecular Ecology at the Max Planck Institute for Marine Microbiology initiated a marine environmental genomics initiative – the real environmental genomics project (REGX) in the year 2000. This project aims at the understanding of the adaptations of marine bacteria to changing environmental conditions.

In marine habitats, Planctomycetes were shown as abundant members, involved in important transformations in the global C- and N-cycles. With 7.145 Mb Rhodopirellula baltica SH1T has one of the largest circular bacterial genomes sequenced so far. The annotation process identified the standard pathways for heterotrophic bacteria like glycolysis, citrate cycle and oxidative phosphorylation. Rhodopirellula baltica SH1T lacks the glyoxylate bypass and the Entner-Doudoroff pathway but exhibits the pentose phosphate cycle. Unexpected for an aerobic heterotrophic bacterium was the presence of all genes for heterolactic acid fermentation, key genes for the interconversion of C1-compounds and 110 sulfatases. The now available blueprint of life for Rhodopirellula baltica SH1T allowed us to predict a certain life-style for this fascinating organism.

Investigations based on proteomics and transcriptomics rise evidence that at least a fraction of the 110 sulfatase genes found in R. baltica are expressed and active. Studies in collaboration with the University in Graz showed even a highly enantioselective sec-alkyl sulfatase activity with retention of configuration when several substrates where tested on resting whole cells of R. baltica. This confirms not only that some of the sulfatases are highly active, it also demonstrates clearly their biotechnological relevance for the conversion of racemates. Taken together, there is growing evidence that R. baltica is a key player in the degradation of complex and sulfated polysaccharides.

Functional genomics II
Thursday, 16:20 – 16:50

Functional genome analysis of the marine bacterium 'Gramella forsetii', a predicted specialist for degradation of polymeric organic matter

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Members of the Bacteroidetes, formerly known as the Cytophaga-Flavobacteria-Bacteroides (CFB) phylum, are among the major taxa of marine heterotrophic bacterioplankton frequently found on macroscopic organic matter particles (marine snow). In addition, they have been shown to represent also a significant part of free-living microbial assemblages in nutrient-rich micro-environments. Their abundance and distribution pattern in combination with enzymatic activity studies has led to the notion that organisms of this group are specialists for degradation of high molecular weight compounds in both the dissolved and particulate fraction of the marine organic matter pool. This implies a major role of Bacteroidetes in the marine carbon cycle, and it seems highly interesting to explore the extent to which the genome contents of members of this group reflect general and special capabilities consistent with their anticipated role in the process of organic matter remineralization.
Recently, we completed the analysis of the first genome (3.8 Mb) of a marine member of the Bacteroidetes, 'Gramella forsetii' KT0803, a North Sea isolate phylogenetically affiliated with the Flavobacteria (Bauer et al. 2006). The predicted proteome disclosed several traits which in joint consideration suggest a clear adaptation of this marine Bacteroidetes representative to the degradation of high molecular weight organic matter, such as a substantial suite of genes encoding hydrolytic enzymes, a predicted preference for polymeric carbon sources and a distinct capability for surface adhesion. Currently, we are establishing a whole genome DNA microarray (70mer oligonucleotide probes, targeting 3588 genes) for comparative expression profiling, to investigate the organism's response to varying nutrient qualities and quantities, and to follow the expression of specific hydrolytic activities.

Reference:
Bauer M, Kube M, Teeling H, Richter M, Lombardot T, Allers E, Würdemann CA, Quast C, Kuhl H, Knaust F, Wöbken D, Bischof K, Mussmann M, Choudhuri JV, Meyer F, Reinhardt R, Amann RI, and Glöckner FO. Genome adaptations of the marine Bacteroidetes member 'Gramella forsetii' for polymeric organic matter degradation (submitted)

Functional genomics II
Thursday, 16:50 – 17:10

International affairs

Chair: Ulrike Lindequist

Stimulation of biotechnology and knowledge transfer by the International Bureau (IB) of the BMBF

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The IB assists the Federal Ministry of Education and Research in the planning and implementation of the international cooperation in research (and education). It supports and advises German institutions (academia, industry) in the planning and implementation of the international cooperation in main research areas and educational policies with selected partner countries. Actually the IB supervises about 1000 running projects (Europe, America, Asia, Africa, Middle East, Turkey). The demands of the IB within the international cooperation can be summarized by (1) strengthening the research and educational landscape, (2) global knowledge/resources, (3) marketing (research, education) and the active formation of the European Research Area (ERA), further on (4) the presentation of the German interests in Europe and the expansion of the cooperation with third states, especially the building of networks and strategic alliances.
The competencies of the IB are based on (1) information and consultation, (2) cooperation potential of the partner countries, (3) general framework for the cooperation, (4) grant programs, (5) the access to decision makers in partner countries, (5) exploration und stimulation of contacts, (6) the support of new cooperation projects and others.

A European Marine Biotechnology Network: A Web of Networks to Promote Open Communication and Innovation?

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Biotechnology can be regarded as a set of technologies which are rapidly changing industry sectors and society. One prominent area for application of biotechnology is in the marine sector. Despite this promise, marine biotechnology is not a major area of application worldwide, possibly excepting the USA, Japan and other rapidly developing countries, mostly in Asia [1, 2, 5]. A current notion of marine biotechnology focus its applications to aquatic activities, whereas other biotechnologies are defined in terms of their end-applications [3]. The marine sector is in a period of significant change: the focus is changing from 'hunting' to 'farming' and 'bioprospecting'; and rapidly emerging technologies are providing information on hitherto unexplored marine resources. A modern definition of marine biotechnology could be: "The use of marine organisms, at the whole, cell, or molecular level, to provide solutions, products or models that benefiting society," (modified from 1, 3). In practice marine biotechnology encompass a broad range of activities. Life originated in the sea and the world's oceans comprise the largest part of the biosphere and contain the most diverse forms of life. Our understanding of life during the last years through the advent of genomics/metagenomics/functional genomics and bioinformatics has opened new perspectives. Perhaps the best description of current activities within marine biotechnology is through a "Vision" (modified from 1): " The application of advanced tools from molecular biology and information technology to a suite of diverse marine habitats and organisms, in order to obtain novel genes, processes and model systems that can be turned into products and approaches for the benefit of industry, research, and to support the sustainable management of the world's oceans."

In Europe a network of well-equipped marine stations and research vessels has been collecting information about marine biota and their biology, driven mainly by the need for resource management and for the study of biological features of marine organisms of commercial or scientific interest. The growing importance of marine science, the rapidly expanding aquaculture industry and associated methodologies make it possible to study and utilize new aspects of marine life. Research activities in marine biotechnology in Europe can thus draw upon an extensive infrastructure and accumulated knowledge concerning marine ecosystems, populations and organisms. Governments and industry have recognized the importance to establish interdisciplinary research focusing on marine biotechnology, and thus scientists have access to a rich variety of marine life from the Mediterranean to the Arctic. Through international cooperation European scientists will also have access to biota and experimental models of all the world's marine resources. With a global market valued at $2.4 billion (2002), and a predicted annual growth rate exceeding 10%, marine biotechnology is a promising sector [3]. It is envisaged that marine biotechnology will contribute to industry sectors from healthcare to bioremediation and from cosmetics to nutraceuticals and food, including applications associated with fisheries and aquaculture. The time has arrived to invest in the underpinning science, knowledge networks, and public understanding of this field. The need for international cooperation in marine sciences is obvious since marine habitats are not restricted to national boundaries. Whereas there are extensive contacts between European groups or individuals, marine biotechnology is not a research area which has been well coordinated in the past. The Marine Board of the European Science Foundation (ESF) described European marine research as "excellent but sub-critical" (in 1). This has called for a European Network promoting dissemination of marine biotechnology discoveries, including industry collaboration. Because of the complexity of scientific collaboration in Europe there is a need for information-providers and science networking. Also there is a need to increase mobility among scientists, in order to better utilize the infrastructures and biological models that are already present. This focus became clear in "The Galway Declaration" [4].

Several European countries have formed plans for increased activities within marine biotechnology, such as Ireland [5]. Other models are focus on Meta-Regions, such as the Scandinavian – Baltic (ScanBalt) networks, also building bridges to medical and other biotechnologies – and using the term "network of networks" to describe their goals of open cooperation and knowledge distribution. Cooperative efforts forming centers of excellence between a range of German Universities also exists, and we find similar efforts in Scandinavia, France and other parts of Europe. Norway has established the highly fucused SARS Centre of Marine Molecular Biology, biodiscovery activities in Tromsø are expanding fast, and Scotland has launched an applications-focused European Centre of Marine Biotechnology. The European Society for Marine Biotechnology (ESMB) was formed in 1994. Since then the ESMB has been involved in more than 10 conferences and workshops focusing on various themes within marine biotechnology. The ESMB has also been involved in promoting marine biotechnology towards policy-makers – including the EU. It has been perceived that the EU has been rather slow in adopting a "science-driven" policy in this area, and some early efforts for international cooperation did not take off [6]. However, formal and informal cooperation does exist, and has also had a forum in the series of International Marine Biotechnology Conferences (IMBC´s), and the recent (2005) formation of an International Marine Biotechnology Association (IMBA). Driven mainly by enthusiasm, these organizations and activities may serve as examples for the wish and need to communicate within and across scientific fields. The viability or effectiveness of these mainly informal networks will depend on their ability to act as information providers between scientists – and towards policy makers and society in general. The enthusiasm that characterizes the marine world as "an ocean of opportunities" is promising for recruitment. The challenge will be to build "networks" which are open and inviting (network of networks) to tap into the vast resources of the seas, take care of these resources for the future – and recruit the best scientists for these tasks.

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International affairs
Thursday, 17:30 – 18:00

Enzymes

Chair: Thomas Schweder

Biocatalysis at low temperatures: a challenge for life

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Life at low temperatures requires a vast array of cellular adaptations that are now analyzed in connection to genome sequence data of psychrophiles. Amongst these adaptations, the so-called thermodynamic challenge refers to the requirement to cope with the exponential decrease of enzyme-catalyzed reaction rates at low temperatures in order to maintain metabolic fluxes compatible with sustained growth. This is achieved by synthesizing cold-active enzymes which however display a pronounced heat-lability of the activity and, generally, of the protein structure. The current views suggest that during evolution, the strong selective pressure for cold-activity and the lack of selective pressure for stable proteins resulted in highly active enzyme catalysts possessing the required structural flexibility at temperatures that tend to freeze molecular motions. Recent studies demonstrate that the active site of cold-active enzymes is more accessible to the substrates. Furthermore, the amino acid side chains involved in substrate binding and in catalysis posses an improved mobility, responsible for cold-activity, and resulting from the disappearance of protein stabilizing interactions far from the active site.

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Accessing novel enzymes from associated bacteria of the marine sponge Aplysina aerophoba

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Sponges constitute one of the most fruitful sources of natural materials among the marine organisms. One reason for this is that they harbour large amounts of endosymbiontic and associated bacteria and cyanobacteria in the mesohyl, which constitutes about 40 % or even more of the total biomass of the tissue. As most of these micro-organisms are not yet characterised, they constitute a huge resource for novel enzyme activities.
Recent projects engaged mainly in the direct isolation of small bioactive compounds rather than proteins. However, access to the natural resources of sponge is limited; they are not fully renewable and highly difficult to cultivate.
We screened bacteria living in a close association or even intracellular in an endosymbiontic relationship with the marine sponge Aplysina aerophoba from the Mediterranean Sea for lipolytic activity by a simple plate assay based on the hydrolysis of tributyrin in a marine media. Several initially grown bacteria couldn't be maintained in liquid media. One of the isolates which grew in liquid and solid media showed highest lipolytic activity among the screened colonies. As it was not yet described before we identified it by rDNA sequencing and some metabolic assays as a Bacillus species. Further micro-organisms enriched belong to Serratia marcescens and Alpha proteobacterium spec.
Screening of a genomic library of the isolated Bacillus strain revealed three clones showing hydrolytic activity toward tributyrin. After sequencing of the heterologous DNA-inserts of ~3000 bps three ORFs of putative hydrolases (two esterases, one amidase) were overexpressed in E. coli, purified by IMAC and characterised biochemically regarding their catalytic properties as well as regarding their structural features and sequence similarities toward other enzymes. Although to be expected for enzymes from marine origin the high salt tolerance of the recombinant enzymes is remarkable. Homologies of those genes towards other known sequences (Genbank) were found below 30 %. In combination with the Bacillus strain described for the first time this emphasizes the high potential of marine micro-organisms. However, cultivation of either sponge or the micro-organisms living in associations seems to be highly difficult if not impossible. Thus, cloning and expression of the metagenome of these micro-organisms seems to be a promising alternative approach to get access to new biomaterials from the marine origin.

Enzymes
Friday, 9:30 – 9:50

Deep-sea Fungi as a source of cold-tolerant proteases

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The deep-sea environment is a source of unique microorganisms with great potential for biotechnological exploitation. Microorganisms living in the deep sea have special features that allow them to live in this extreme environment, and it seems likely that further studies of these organisms will provide important insights into the origin of life and its evolution. The deep-sea inhabitants being present in extreme conditions produce extracellular enzymes, which are active under such conditions. Amongst the extracellular enzymes produced, proteases are important class of enzymes, which occupy a pivotal position with respect to their physiological role. Proteolytic enzymes play an important role in remineralisation processes in the sea, mainly because proteins and peptides constitute a substantial portion of the organic nutrients present in the deep-sea sediments as well as suspended particulate matter. Apart from their ecological significance, proteases are one of the most valuable commercial enzymes. More than 25% of the worldwide sale of enzymes is contributed by proteases alone, where mainly alkaline proteases are used. In our study, a total of 221 deep-sea isolates of fungi from 5000 m in the Central Indian Basin were screened for the production of protease enzyme. Aspergillus ustus (NIOCC#20) producing the highest amounts of the enzyme was selected for further studies. The fungus produced a maximum of 1,639 ACU mL-1 of protease by day 7. The enzyme, with molecular mass of 32 kDa showed several interesting properties. It had a broad pH range of 6 to 10, with an optimum at pH 9. The optimum temperature for protease activity was 45°C and approximately 10 % of the activity was retained at 2°C. The enzyme was totally inhibited in the presence of 2 mM PMSF suggesting it to be a serine protease. It was active in the presence of several commercial detergents at 2 g L-1 concentration and in the presence of 0.5 M NaCl, equivalent to 29 parts per thousand salinity. In the presence of stabilizing agents such as glycerol, CaCl2 its thermostability at 60°C was enhanced. Heavy metal ions copper, Hg, Fe, Ni and Zn did not inhibit the enzyme activity considerably. This isolate produced 2500 ACU ml-1 of alkaline protease when grown by solid substrate fermentation in defatted groundnut oil meal after 48-72 h of incubation at 30°C. This study indicates that fungi from deep-sea sediments could be a useful source of proteases and could be used for commercial preparations using low cost substrates.

Enzymes
Friday, 9:50 – 10:10

Extremophiles: from genomes to enzymes

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Before the "genomics era" the enzymology of prokaryotic microorganisms was studied mainly via biochemical and genetic approaches, and genes encoding enzymes of interest for fundamental studies or for biotechnological applications were normally isolated from gene libraries constructed from defined, cultivated strains of microorganisms. Since then, additional strategies have become available which are based on the use of complete or partial genome sequence information of selected organisms to mine for enzyme genes. Another approach with large potential is to tap the immense but often largely uncultured biodiversity present in microbial communities found in natural habitats with metagenomics and high-throughput screening techniques. The focus of this presentation will be on work from our lab dealing with enzymes from Thermotoga maritima, a hyperthermophilic marine bacterium growing at temperatures up to 90°C, and from the thermoacidophilic archaeon Picrophilus torridus, an organism capable of growth at 65°C around pH 0.

Enzymes
Friday, 10:40 – 11:10

Tools for high-throughput screening and optimization of biocatalysts

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An impressive number of examples has been developed in the past decades for the application of enzymes in organic synthesis, with hydrolases as the most frequently used biocatalysts[1].
Whereas initially, commercial enzyme preparations have been discovered by classical screening, the current trend is to get access to novel biocatalysts from the metagenome. However, this results in a large number of positive hits and consequently the identification of enzymes suitable for a given application can be very time consuming, if only standard analytical methods such as GC or HPLC are used. In addition, the method of directed evolution[2] also results in large mutant libraries representing a similar challenge in biocatalyst identification. We have therefore developed in the past few years several high-throughput screening methods to facilitate the identification of best candidate biocatalysts.
This includes several assays in the microtiter plate scale to identify stereoselective lipases and esterases using hydrolytic[3] and synthesis activity[4] assays, a screen for amidase activity[5], a growth assay[6] and a polarimetric assay[7]. For proteases, we recently developed a phage display system, which allowed us to determine the substrate specificity of the proteases Subtilisin and the outer membrane protease T (ompT)[8].

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Enzymes
Friday, 11:10 – 11:40

Marine proteomics

Proteomics of marine microorganisms

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Microorganisms from marine habitats are a promising source of new pharmacologically active components. Furthermore, because of their special living conditions and functions in the ecosystem, marine microorganisms have developed a variety of unique metabolic pathways that can be put to work in different biotechnological applications. Since the majority of the ocean water is relatively cold, most marine bacteria are cold adapted and are therefore a good source for the search of cold adapted enzymes.
The recent development of genome sequencing made many bacterial genomes available for research, among them several genomes from marine bacteria. But the genome sequence represents only the blueprint of life demonstrating the metabolic capacities of an organism. With the means of transcriptomics and proteomics, however, the analysis of the genes actually expressed during growth or in response to different environmental stimuli such as nutrient starvation is possible. This allows the exploration of metabolic pathways used by different microorganisms under specific environmental conditions. We present here data of proteomic studies of the marine bacteria Rhodopirellula baltica and Pseudoalteromonas haloplanktis. Using 2D gel electrophoresis the cytoplasmic as well as the periplasmic and cell wall proteome were analysed providing insights into the physiology of the microorganisms during growth and in response to unfavourable environmental conditions.

Marine proteomics
Friday, 11:40 – 12:00

A marine proteome database

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We present an information system for the functional analysis of proteomes of marine microorganisms. The Marine Proteome Database leverages Protecs, a web-based information system for functional genomics, to support internal lab workflows, collaboration between different sites, as well as publication of results. Using the system researchers can share the data generated from multiple projects and collected from external sources, such as proteome maps, expression data, raw images, and sample information. The tight integration of lab data (sample descriptions, genomes and proteomes, 2D gels, MS data, DNA arrays) with analysis applications for 2D gels and peptide mass fingerprinting provides the necessary infrastructure for effective information management in a distributed and highly collaborative research environment.

Marine proteomics
Friday, 12:00 – 12:20

P1

Marine Fungi As An Unconventional New Source Of Bioactive Natural Products

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Interest in metabolites produced by marine fungi was not apparent until 1948, as a result of discovery of the antibiotic cephalosporines(1). However investigations have been intensified more recently (1977) due to searching into new sources for bioactive metabolites which led to the discovery of the first marine representative of the dioxopiperazine alkaloids, gliotoxin, a very promising antibacterial and antiviral agent. Another interesting discovery for medical applications was that of phomacetines, a new class of platelets activating factors (PAF) antagonists which constitute a promising pharmacological tool and a new entity in this field. Fellutamide A and epolactaene, two different neurotrophic agents of distinct chemical structures, reflect a new area of interest in chemical and pharmaceutical research. They confirm marine fungi as producer of a vast number of biologically active metabolites with new types of structure because of their living conditions and functions in the ecosystem. This potential has led us to investigate cultured marine derived fungi obtained from the inter-tidal zone of the Red Sea, in the region between Hurgada and Safaga for bioactive metabolites. Twelve different isolated strains have been cultured, extracted and their biological activity as antibacterial and antifungal has been assisted. Comprehensive chemical investigation of the first detected bioactive extract was presented here. This work is a part of an ongoing research cooperative project between the NRC and University of Greifswald, Germany.

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P2

Characterization of cold adapted neutral halophilic proteases from deep-sea bacteria and their antifouling properties

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Six deep-sea bacteria isolates from the Aleutian margin (Alaska, USA) were screened for the production of proteases. The effect of the proteases on the attachment of the bryozoan Bugula neritina was investigated. The bacteria which produce proteases belong to Pseudoalteromonas spp. Of the six proteases, two were neutral cold-adapted proteases that showed the activities at an optimum pH 7-8 and an optimum temperature close to 35°C, and the other four were alkaline proteases that showed the activities at an optimum pH 9 and an optimum temperature of 40-45°C. Partially purified proteases from 6 deep-sea species significantly decreased attachment of B.neretina at concentrations of 0.03 - 1 mIU ml-1. One cold-adapted neutral halophilic protease produced by P. issachenkonii UST041101-043 (GenBank code DQ178021) was purified to electrophoretic homogeneity and its molecular mass by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was 34kDa and by ESI-MS was 32411 Da. This protease showed its optimal activity at a sodium chloride concentration of 2 M. Neither phenylmethyl sulfonylfluoride (PMSF) nor ethylenediaminetetraacetic acid sodium salt (EDTA-Na) could inhibit the activity of this protease. De novo amino acid sequencing proved this protease to be a novel protein. The EC50 of antifouling effect of the pure protease was 0.1 mIU ml-1 (~ 1 ppb). The protease incorporated in a water-soluble paint significantly inhibited biofouling in a field experiment. Our investigation demonstrated the potential use of proteolytic enzymes for antifouling defense.

P3

New Marine Gliding Bacteria: Potential Sources of Cytotoxic Compounds

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The search for cytotoxic compounds from microorganisms has been focusing mostly on the well studied taxonomic groups such as fungi, actinomycetes and other soil bacteria. Myxobacteria have been reported to produce chemically diverse classes of cytotoxic compounds such as apicularens A and B, tubulysins A-E, rhizopodin, chondramide A and epothilon A. However, neoverrucosane diterpenoids is the only known natural product isolated from marine gliding bacterium Saprospira grandis. In this study, we attempted to exploit one of the least studied groups of microorganisms known as gliding bacteria from marine habitats in Thailand in order to investigate their potential as the producers of cytotoxic compounds. The results show that marine gliding bacteria are capable of producing the extracts which showed potent cytotoxicity against 4 human cancer cell lines including HeLa, MCF-7, HT-29 and KB depending on the composition of cultivation media. The analysis of nucleotide sequences of 16s rRNA of the active strains suggested that 7 isolates should belong to the new genera in the phylum Cytophaga-Falvobacterium-Bacteroides(CFB) whereas the others are the related to Tenacibaculum mesophilum and Saprospira species.

P4

Computer-Aided Test and Selection for Lycopene β-cyclase Inhibitors: Molecular docking of structurally diverse herbicidal inhibitors in Dunaliella salina CCAP 19/18

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Lycopene has long been recognized for its antioxidant property. It is also of great interest for its regulatory effect on cell growth and may also protect humans against certain disorders such as prostate cancer. β-Carotene is a lycopene metabolite which contains beta-ionone rings at each end of the molecule, is naturally found in plants e.g. tomatoes, via the action of the enzyme lycopene β-cyclase. Some herbicides inhibit lycopene β-cyclase and eventually suppress lycopene conversion to β-carotene. To assess the β-cyclase activities of herbicides we carried out a molecular docking study on a set of twelve structurally diverse herbicide compounds. We compared the effects of herbicides containing amine group on potential inhibitory of lycopene β-cyclase in the microalgae, D. salina CCAP 19/18. This newly recognized mechanism in herbicidal activity is also the basis for the mode of action of other lycopene β-cyclase inhibitors as well as phytoene desaturase inhibitors. Molecular modeling studies are useful to investigate the molecular mechanism of these herbicides which directly prove the inhibition site by correlating the structure of chemically modified inhibitors with their inhibitory activity. The effects of cyclic amine compounds on lyconene production by D. salina CCAP 19/18 have been investigated and their Ki have been compared with other herbicides. Molecular modeling data and analysis of lycopene accumulation in D. salina CCAP 19/18 showed that amitrole and also nicotine (a non-herbicide β-cyclase inhibitor) could be considered the most potent lycopene β-cyclase inhibitors.

Keyword: Dunaliella salina CCAP 19/18, Lycopene β-cyclase, Docking, Amitrole.

P5

Metagenomic analysis of secondary metabolite gene clusters from marine sponge-associated microbial consortia

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Marine sponge-associated microbial consortia are widely recognized as promising sources for novel pharmacologically important secondary metabolites. However, access to this natural resource has been limited by the fact that these microorganisms have not been cultivated so far. In order to explore the diversity of secondary metabolite gene clusters of the microbial community associated with the sponge Aplysina aerophoba, a metagenomic library using an E. coli - Streptomyces shuttle vector was constructed. Gene clusters encoding for type I polyketide synthases (PKS-I), non-ribosomal peptide synthases (NRPS) and putative microcin-related genes were identified. Complete sequence analysis of a selected cosmid clone revealed the presence of a sponge-specific PKS-I operon, probably encoding for the biosynthesis of methylated fatty acids, as well as novel putative ORFs with unknown or hypothetical functions. A cosmid clone containing gene encoding for a putative microcin-related protein homologous to PatD was identified and partially characterized. PatD is believed to be involved in the cyclization of amino acid residues and shears similarity to adenylating enzymes. Several PatD homologs have been identified in different biosynthetic clusters. Experiments for heterologous expression of the identified gene clusters are ongoing. Our study provides insights into the biosynthetic potential of the so far uncultivated marine sponge-associated microbiota and underlines the importance of metagenomics as a promising strategy for sustainable production of novel secondary metabolites.

P6

Diversity assessment and biotechnological exploration of sponge-associated bacteria: Molecular biological approach

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Marine organisms are well known to have a specific relationship with numerous microorganisms and sponges are not exception to this. Scientists have been fascinated by the secret of sponge-bacteria association since long time. The studies on this association are highly appreciated due to the importance of marine sponge as living fossil as well as its significance in drug discovery. In the present study, bacteria were isolated and cultured from different species of marine sponges, having same ecological habitat. Phylogenetic analyses of these bacteria were carried out by using 16S rRNA gene sequences. Bacterial PCR products were digested with four-base-cutting restriction enzymes for RFLP studies, in order to evaluate the degree of polymorphism existing among these strains. It was observed that the different sponge species from same ecological niche showed diversity in their associated bacterial population except some specific association of alpha-proteobacteria with sponges. These bacterial isolates were further explored for their biotechnological potential by detecting NRPS and PKS gene clusters, which are involved in the synthesis of large structurally diverse bioactive metabolites of bacterial origin. It is important to note that some of the sponge-associated bacteria were found to exhibit this kind of genetic machinery for the production of secondary metabolites. In summary, comprehensive molecular biological investigations of the sponge-associated bacteria highlight the biotechnological importance of these bacteria with special emphasis on their diversity and specificity with the host sponges.

P7

Enzymes for the engineering of marine bacterial exopolysaccharides

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In recent years, there has been a growing interest in the isolation and identification of new microbial polysaccharides with potential uses in many industrial sectors. Deep-sea hydrothermal vents offer a valuable biodiversity which makes these ecosystems a rich source of new microorganisms able to produce exopolysaccharides of biotechnological interest. New exopolysaccharides (EPS) with high molecular weight (around one million g/mol) have been identified. They are usually branched and heteropolymeric with a complex repeating unit. Most of them are composed of neutral and acid sugars. But one linear exopolysaccharide containing hexosamines has also been found. These EPS are also slightly substituted by acetate, lactate, pyruvate or sulfate [(1-4)]. Their biological activities are based on molecular features including molecular size as well as osidic residues, linkages and substituents. Chemical or enzymatic engineering of these polymers would maximize their applications and generate new biological functions in different industrial fields such as environment, pharmacology (cancer research, therapeutic angiogenesis, bone healing.), dermatology, cosmetic products and medical imaging. In all cases, the degree of polymerization, N-acetylation and sulfation of the derivatives proved to be extremely important for bio-activity. Low molecular weight exopolysaccharides can be generated by acid hydrolysis or free radical depolymerization. N-deacetylation and sulfation are also carried out by chemical methods. However, enzymatic methods meet well the need of better control of the modification process. The conditions of enzyme catalysed reactions are generally softer than those in classical chemistry. Enzymes are highly selective allowing control of final product characteristics.In the purpose to develop therapeutic compounds, we are looking for new enzymes to produce EPS derivatives. Enzymes able to carry out targeted modifications on degree of polymerization or on functional substitutions may be obtained by screening of enzyme extracts or commercial preparations having a broad substrate specificity; purification from an entire growing microorganism, or by directed evolution of existing enzymes to increase efficiency or modify substrate specificity. Results on the screening and study of depolymerizing and N-deacetylating enzymes able to modify HE800 exopolysaccharide[(3)] from marine Vibrio diabolicus[(5)] will be presented.

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P8

Metagenome-derived Anti-Quorum Sensing Molecules

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Metagenomics is the investigation of the genomic potential of a habitat by direct DNA extraction, cloning and expression in a heterologous host. This approach has been used to find many novel biocatalysts and molecules with widespread applications. The ability of microorganisms to coordinate regulation of their genes in relation to cell density, quorum sensing, was first elucidated in the marine microorganism Vibrio fischeri. In pure culture studies quorum sensing has been shown to regulate many aspects of biofilm formation. Anti-quorum sensing, the disruption of the quorum sensing signalling interaction, has gained a lot of interest recently as a potential mechanism for preventing formation of disadvantageous biofilms in natural and clinical environments. Whereas analysis of quorum sensing relies on pure culture experiments, the detection of molecules capable of quorum quenching can be achieved by molecular methods such as metagenomics. The majority of anti-quorum sensing molecules in the environment may be retained within the uncultured fraction of the prokaryotes. The metagenomic approach makes it possible to express and detect these molecules in a heterologous host. Despite the fact that quorum sensing is normally species specific, it is possible to find anti-quorum sensing molecules which disrupt biofilm formation in more than one species. This is an important consideration when developing anti-quorum sensing as a strategy for combatting biofilm formation in a wide range of environments. When a dual screening process was used to detect anti-quorum sensing molecules in a metagenomic library it revealed the presence of 2 clones capable of inhibiting the swarming phenotype, involved in biofilm formation, of both Pseudomonas aeruginosa and Escherichia coli. While the mechanism of anti-quorum sensing activity in these clones remains to be determined, these initial experiments indicate that the anti-quorum sensing molecules involved can be used to disrupt biofilm formation in more than one clinically and environmentally important bacterial genus.

P9

Biochemical characterization and structure analysis of extremophilic isocitrate dehydrogenase

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In order to analyze thermal adaptations comparatively within one protein family, we have chosen isocitrate dehydrogenase (IDH) as model enzyme. Biochemical characterization of IDH from psychrophilic (Desulfotalea psychrophila, DpICDH), mesophilic (Desulfitobacterium hafniense, DhIDH), moderate thermophilic (Clostridium thermocellum, CtIDH), and hyperthermophilic (Thermotoga maritima, TmIDH) micro-organisms has been performed. TmIDH was most thermostable with a melting temperature (Tm) of 98.3 °C [(1)]. A high Tm of 67.7 °C was determined for the psychrophilic DpIDH, a value similar to CtIDH (Tm = 67.9 °C), but higher than for mesophilic DhIDH (Tm = 58.6 °C). Although showing high global stability, DpIDH showed lowest optimal temperature for activity (Topt) with a broad activity optimum at 35-40 °C. The Topt of DhIDH, CtIDH and TmIDH was 45, 70 and 90°C, respectively. By implication, the psychrophilic DpIDH reached its maximal enzyme activity far below its Tm whereas there was a close correlation between these two parameters for the mesophilic and (hyper)thermophilic IDH investigated. Furthermore, when Km[isocitrate] and Km[NADP] were determined in a range between 5 and 45 °C, a significant increase in Km[isocitrate] was observed for DpIDH when the temperature exceeded the optimum growth temperature of D. psychrophila (10 °C). Km[NADP] was less effected by temperature. An increase in Km[isocitrate] was also observed for DhIDH, but occurred at a higher temperature and the effect of temperature was less pronounced. Km[isocitrate] was only slightly increased for the thermophilic CtIDH and the temperature effect on Km[NADP] was less than on Km[isocitrate] for both DhIDH and CtIDH. The kinetic data obtained indicate that the binding site geometry of DpIDH, especially the isocitrate binding site, is disrupted at lower temperatures than the other IDHs included in this comparative work. Hence, the cold activity in DpIDH is probably achieved through local flexibility in the active site region.
We have recently solved the structure of TmIDH[(2)] and DpIDH and the biochemical data obtained will be discussed in a structural context focusing on of cold-adaptation of DpIDH and heat adaptation of TmIDH.

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P10

Establishment of marine marcophytic cell cultures for the production of bioactive metabolites

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Marine algae are the largest producers of biomass in the marine environment. They produce a wide variety of chemically active metabolites in their surroundings, thus macrophytic marine algae are a rich source of unique bioactive compounds. These active metabolites have all sorts of pharmaceutical activities like antimicrobial, antifouling, antioxidant, cytotoxic and anti-inflammatory properties. But most of these bioactive substances were isolated of algae which were collected from natural habitats. The Cell Cultures of macroalgae such as Ulva sp. or Gracillaria sp. would open up new possibilities for increased production of potential pharmaceutical substances under well-defined conditions without debility of natural resources. The anatomically complex macroalgae must be introduced into liquid single cell suspension culture by using different methods of callus induction or protoplast isolation. For this purpose it is necessary to find a practical sterilization to get axenic vital algae cultures. Different examinations showed that a multilevel treatment with betadine is particularly suitable to obtain tissue completely free from biological contaminants (bacteria, fungi and algae). By this axenic tissue callus induction will be research depending on a change of media and light. Furthermore different bioreactors (Wave reactor, bubble column, shaking flask) are under examination in influence on the callus induction. With a specially developed screening system the isolated metabolites are examined for their biological activities.

P11

Alteration of secondary metabolite profile of fungi in response to a competitor's challenge.

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Some of the biosynthetic pathways in fungi that are responsible for secondary metabolite production are silenced under standard cultivation conditions. This has been ascribed to missing environmental challenges. Given that many secondary metabolites have evolved in the process of natural selection as defensive compounds, it is plausible that the expression of these dormant genes can be induced by the presence of other fungi.
To check this hypothesis, four fungal strains producing cytotoxic and/or antifungal compounds were grown in mixed cultures. Every strain was first grown in triplicate and, after establishment of a stable culture, each of these three cultures was then"infected" separately with small cultures of the other three strains. The mixed fermentations were continued for two more weeks. A pure culture of all four strains was grown for comparison purposes. After extraction, the secondary metabolite pattern of the mixed and pure cultures were analysed by HPLC-UV, HPLC-MS and cap probe NMR.
In this communication the chemical profile of the "killers" and "survivors" and their probable defensive and attacking chemical arsenal will be presented.

Acknowledgments

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P12

Developing a sustainable supply of 1,3-alkylpyridium salts for use as novel drug delivery agents from the Mediterranean sponge Reneira sarai.

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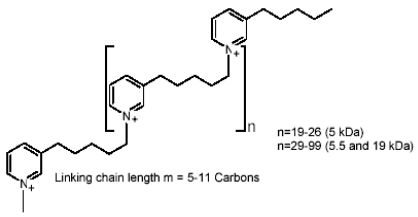
Marine sponges are sessile filter feeders that have developed a variety of spectacular strategies for survival over the last 500-700 millions years. Sponges have the ability to produce a large variety of chemical defences which prevent over-predation, defence against competition from foreign microorganisms or changes in environmental conditions.
Of the many distinct chemical weapons produced by sponges a number are toxins including the unusual oligomeric 1,3-alkylpyridinium salts (1,3-APS) that have interesting biological properties including pore forming effects that we would like to exploit for future gene and peptide drug delivery systems(1). As chemical synthesis of monodisperse 1,3-APS oligomers has not yet been achieved, we propose to attain this by the use of heterologous expression of the relevant biosynthetic pathway using metagenomic DNA from the Mediterranean sponge Reneira sarai extracted from tissue provided Dr Kristina Sepèiæ and Prof Tom Turk of the Department of Biology, University of Ljubljana, Slovenia.



oligomeric 1,3-alkylpyridinium salts

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P13

Structurally Diverse New Metabolites from New Zealand Marine-Derived, Entomopathogenic and Endophytic Fungi

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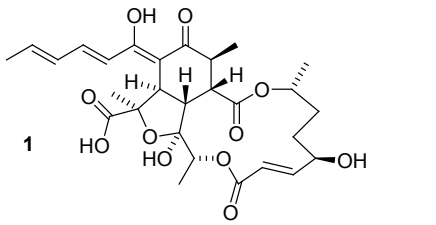
In screening filamentous fungi for new and biologically active metabolites it is of particular importance to find ways of avoiding the isolation of known natural products. Our project, aimed at the isolation of cytotoxic and antimicrobial secondary metabolites from New Zealand fungi, dealt with this problem by focusing on fungi from specific ecological niches and by a powerful dereplication system.
The fungal strains investigated belonged to three specific ecological groups:

- fungi isolated from marine invertebrates, algae, and driftwood
- entomopathogenic fungi isolated from infected insects and other arthropods
- endophytic fungi from New Zealand plants.

All fungal isolates were grown as small-scale cultures, extracted and tested for cytotoxic activity. Active extracts were subjected to our dereplication system based on HPLC-MS and -UV as well as UV libraries and natural product databases. Fractionating the small-scale extracts under analytical HPLC conditions into microtitre plates made it possible to assign bioactivities to specific peaks in the HPLC and even to obtain 1H NMR spectra (and even COSY and TOCSY spectra) of single wells using a capillary probe. These NMR data greatly facilitated the identification of known compounds, especially in combination with the database AntiMarin (a combination of AntiBase and MarinLit), which has searchable NMR features included in its datasets.



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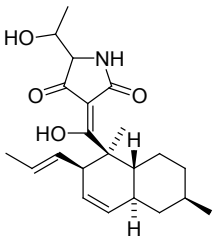

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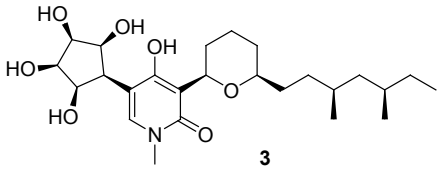

3

Any unidentified bioactive metabolites were isolated for structural elucidation and characterization of the biological activities. New metabolites thus identified included gliocladiolide (1) from a marine-derived Gliocladium sp., paecilosetin (2) from the insect pathogen Paecilomyces farinosus, and the new funiculosin derivative 3 from an unidentified endophytic fungus.

P14

A new myxobacterial metabolite: Isolation and structure elucidation

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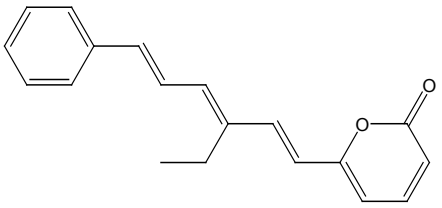
Myxobacteria are gram-negative rod shaped bacteria commonly found in soil. They form swarm-like colonies which spread over surfaces and feed on other microorganisms. The most outstanding feature of myxobacteria is their ability to form fruiting bodies by which the different genera can be distinguished.
In the last years myxobacteria have been in the focus of natural products research and have become known as potent producers of secondary metabolites. To date more than fifty unique structural types have been isolated, among them compounds with promising biological activities, e. g. the epothilones which are now in clinical studies as anticancer drugs. The structure of the epothilones shows striking similarities to peloruside A, a sponge metabolite [(1)]. Apart from the epothilones there are other myxobacterial metabolites which resemble marine metabolites, e. g. the chondramides which are related to jaspamide [(2)]. Myxobacteria were also reported to produce derivatives of the known marine compound bengamide [(3)].
In our screening program the myxobacterial strain 150 (morphologically characterized as a Polyangium or Nannocystis sp.) was singled out due to the results of TLC, LC-MS and NMR analyses. The next step was the cultivation of this strain in a large scale and subsequent extraction and fractionation. HPLC separation eventually yielded compound 150E, a new metabolite with an unusual ethyl residue connected to a polyunsaturated carbon chain. The structure was elucidated by applying mass spectrometry and different 1D- and 2D- NMR techniques.
Future work will adress the biosynthesis and evaluation of the bioactivity of the compound.



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P15

Utilizing the pharmaceutical potential of Marine Microrganisms

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Microbial natural products are the predominant source for lead structures in the pharmaceutical industry. A high proportion of marine microorganisms form biological active substances and the enormous potential of marine microorganisms remains still largely untapped.
The Zentrum für Marine Wirkstoffe (Center of Marine Natural Products) at the IFM-GEOMAR in Kiel is focussed on the identification of new natural products from marine sources and the investigation of their biological-ecological function. The Center combines microbiological, biotechnological, genetic, and chemical expertise to identify the biotechnological and pharmacological potential of new natural products and to promote them into application. Valuable sources are marine bacteria and fungi isolated from promising habitats, in particular those associated with animals and macroalgae, but also unique collections of more than 10000 marine bacteria and fungi. A test panel of various biological activities in cell-free as well as in cell-proliferation assays is applied. Antimicrobial, antitumor, and antiviral activities are major targets. Chemical analysis by HPLC-MS and bioassay-guided isolation enables rapid identification of bioactive compounds. Actual results will be presented.

P16

Expression profiling of the marine bacterium Rhodopirellula baltica SH 1T

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Rhodopirellula baltica SH 1T is a marine representative of the globally distributed and environmentally important bacterial phylum Planctomycetales. It is a model organism for aerobic carbohydrate degradation in marine systems, where polysaccharides represent the dominant components of biomass. R. baltica shares with other Planctomycetes several structurally unique properties, such as a peptidoglycan less proteinaceous cell wall and intracellular compartmentalization, and undergoes morphological changes during its life-cycle such as budding and cell aggregations.
Within the framework of the REGX-project (Real Environmental Genomics), the complete genome sequence of R. baltica has been determined and functional annotation was performed. However, pure in silico predictions leave about 60% of the genes without functional assignments.
To obtain information about the possible functions and ecological relevance of this huge fraction of hypothetical and conserved hypothetical genes, a whole genome DNA-microarray for R. baltica was designed and established. 70mer oligonucleotide probes targeting 7,377 genes are spotted in three replicates on aminopropylsilane coated glass slides.
Current experiments are focusing on the comparative expression analysis of different life cycle stages of R. baltica. A special emphasis is placed on the expression regulation of the remarkably high number of 110 sulfatase genes present in the genome. It has been assumed that the encoded proteins function in the degradation of sulfated glycopolymers. However, some of them might also be involved in the remodelling of cell wall components during the life cycle, in which case they should show growth-dependent expression patterns.

P17

Antiangiogenic potential of marine sponge-associated bacteria

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Sponges are regarded as microbiological fermenters that hold an untapped potential for natural product drug discovery. In the present study, bacteria associated with the sponge Dysidea avara were isolated, cultured and further identified using 16S rRNA gene sequences. The extracts obtained from these bacteria were screened for antimicrobial activity, anti-angiogenic activity as well as cytotoxicity. Antiangiogenesis studies were carried out using chick chorio-allantoic membrane (CAM) assay and endothelial cells tube formation assay. In this investigation, some of the bacterial strains showed antimicrobial activity and cytotoxicity against PC12 and HeLa cells. Interestingly, the extracts of three bacterial strains from D. avara were potent angiogenesis inhibitors at 5 and 10µg ml^{-1} concentrations. The active compound was isolated from an alpha-proteobacterium using gel filtration on Sephadex LH-20 and reverse-phase preparative HPLC in a bioactivity-guided manner. The compound thus obtained was readily identified using MS and NMR spectroscopy as 2-methylthio-1,4-naphthoquinone (MTN). This compound showed inhibition of cell proliferation of vertebrate tumor cells at a concentration of 80 ng ml^{-1}. However, only 25 ng ml^{-1} of the compound was required to potentially inhibit angiogenesis in CAM assay. MTN was found to be active in endothelial cell tube formation assay also. In summary, this investigation explores the importance of sponge-associated bacteria as a valuable source of novel antimicrobial and anti-angiogenic molecules.

P18

Exploring the proteome of the planctomycete Rhodopirellula baltica

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Living in a changing environment, marine microorganisms show various strategies to physiologically tolerate starvation conditions. The marine planctomycete Rhodopirellula baltica is of special interest because it contains in its genome sequence many putative and unique metabolism genes, such as a high number of sulfatase genes and all genes for heterolactic acid fermentation. Based on the available complete genome sequence, we analyzed the R. baltica proteome by applying different protein as well as peptide separation techniques (1D and 2D electrophoresis and HPLC separation) prior to mass spectrometric analysis. To provide a basis for physiological studies we first created master gels of the intracellular proteome. The current R. baltica proteomic dataset (with substantial contributions from the MPI Bremen) consists of 1021 unique proteins (accounting for 14% of the total putative protein-coding ORFs), including 214 proteins with a predicted signal peptide. A public database (MarProtecs) that comprises the whole genomic and functional genomic information of R. baltica individual proteins is going to be constructed. This database will support the exploration of R. baltica adaptation networks that define the genes induced by single stress or starvation stimuli. Although many bacteria species secrete extracellular proteins in response to nutrient starvation conditions, the marine bacterium R. baltica shows almost no protein secretion into the extracellular medium. Therefore we analyzed the cell wall proteome of R. baltica. The cell wall subproteome was compared to the intracellular proteome showing that a unique protein family containing several YTV domains and that is rich in cystein and proline is a component of the R. baltica proteinaceous cell wall. Furthermore, some initial results of the R. baltica physiological response to nutrient starvation are shown.

P19

Investigation of cytotoxic substances of Vietnamese cyanobacteria

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Fourteen strains of Vietnamese cyanobacteria were cultivated to produce four kinds of extract from both dried biomass (n-hexane-, methanol- and water-extract) and culture medium (ethyl acetate-extract). A total of 56 extracts were screened for cytotoxic/cytostatic activity against three human cell lines: MCF7 (breast cancer), 5637 (urinary bladder cancer) and FL (amnion). Twenty-five extracts including 9 methanol, 7 ethyl acetate and 9 hexane extracts exhibited cytotoxic activity at concentration below 500 µg/ml. The methanol extract from strain VN14 (Oscillatoria spp.) was further investigated to isolate the bioactive compounds. From this extract, two compounds were purified, based on their cytotoxic activity. Interestingly, these compounds also show antimicrobial activity, even much more than cytotoxic activity.

P20

Genome and Proteome Characterization of the Psychrophilic Flavobacterium Phage 11b

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The genome of the lambda-like bacteriophage 11b, which infects a psychrophilic Flavobacterium isolate from Arctic sea-ice, was determined to be a double stranded circular DNA molecule of 36,012 bp with no detectable physical ends. Its GC content corresponds to the recognized low chromosomal GC of host-genus species and represents the lowest GC content of all phages of Gram-negative bacteria sequenced so far. Strong similarity to 'mesophilic' non-marine lambda-like phages, e.g. to bacteriophages SPP1 and HK97, was observed for several of the 65 predicted ORFs and for the genome organization. Early genes presumably encode an ERF, SSB, endonuclease, a methylase, and a primase or transposase protein. The late gene segment is likely to start with a terminase, portal, minor head, protease and a major capsid gene and ends with the lysis module. Fife ORFs exhibited similarities to Bacteroidetes species and seem to reflect the host specificity of the phage. A similarity tree was calculated by means of a global BLASTP analysis in which SPP1 turned out to be the most closely related phage. Virion proteins were separated with PAGE and identified by MALDI-TOF-MS. Among identified proteins are the portal, the major capsid, and a putative conserved tail protein. The genome of 11b is the first to be described of a cultivated virus infecting a psychrophilic host as well as a Bacteroidetes bacterium.

P21

Biological investigation of marine fungi for prevention of viral and bacterial fish diseases

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The antiviral activities of eight species of marine fungi from Baltic Sea were examined against the fish viruses of Infectious Pancreatic Necrosis (IPN) and Infectious Hematopoietic Necrosis (IHN). Ethanolic and aqueous extracts were tested. The ethanolic extracts of Stagonospora sp. and Halosapheira viscosa showed antiviral activity against INP-virus.
Furthermore ethylacetate, dichlormethane and ethanolic extracts of 16 species of marine fungi isolated from Red Sea (Egypt) were tested for antibacterial activity against six fish pathogenic bacterial strains (Flexibacter maritima, Pseudomonas anguilliseptica, Vibrio anguillarum, Aeromonas salmonicida ssp. salmonicida, Aeromonas hydrophila ssp. hydrophila and Yersinia ruckeri). Flexibacter maritima and Pseudomonas anguilliseptica were the two most susceptible bacterial strains. 17 of 34 extracts exhibited a strong to moderate antibacterial activity against Flexibacter maritima.

P22

Cyanobacteria as potential producers of biogenic agents for the growth inhibition of microfouling organisms

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Introduction: Marine biofouling is one of the most important problems currently facing marine technology. This fouling increases surface corrosion and causes several technical problems on ships and other marine infrastructure. For prevention of this marine biofouling antifouling paints were used containing organotin (like tributyltin = TBT), copper and organonitrogen compounds. The worldwide application of TBT-based paints had caused a growing pollution of environment and in 2003 the International Maritime Organization imposed a complete ban for the application of TBT. New antifouling agents had to be developed as possible replacements for organotin compounds. The isolation of antifouling agents from marine organisms seemed to be the most promising and effective method. The aim of our research work was a screening of different strains of cyanobacteria, known as important producers of active compounds for inhibitory activity against the diatom Nitzschia pusilla in comparison to the inhibitory activities of commercial antifouling compounds. Material and methods: There were prepared 49 crude extracts from various strains of cyanobacteria using solvents of different polarity (TBE, n-hexane, methanol, water). These extracts were tested in agar diffusion tests against the diatom N. pusilla, a common microfouling organism. Commercial antifouling agents such as the isothiazolone Sea Nine 211 and the herbicides Diuron and Irgarol 1051 were tested as reference. The lowest concentration at which N. pusilla did not grow was taken as the minimum inhibitory concentration (MIC). Results and conclusions: The MIC values for the TBE extracts turned out to be the most effective ones. These values with the most inhibitory activities were as follows: · 0.0002 mg/ml (from Scytonema hofmanni) · 0.2 mg/ml (from Lyngbya spec.) · 0.2 mg/ml (from Bio 29) Whereas the antialgal activity of S. hofmanni caused by cyanobacterin was already known, antialgal activities of the other cyanobacteria have not been described so far. For practical use the antifouling activity and environmental toxicity of the extracts have to be evaluated in laboratory antifouling assays and in field experiments with stationary and mobile test surfaces by embedding the extracts in different non-poisonous paint-matrices. In addition the isolation and characterization of the active antialgal substances of Lyngbya spec. and the strain Bio 29 is an important task for further investigations.

P23

The isolation of bioactive compounds from tropical cyanobacteria

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Cyanobacteria are well recognized as a potential source of diverse secondary metabolites with antibiotic, cytotoxic and enzyme inhibition activity. The natural products from this group of microorganism have a broad diversity in the type of chemical structure (lipopeptides, amino acids, fatty acids and macrolides) and also in bioactivity (anticancer, cytotoxic, antibiotic). Commonly, the most of active compounds from cyanobacteria are lipopeptides and fatty acids, the polyketide metabolites are less frequently isolated but they possess unique structure. With the aim of seeking antimicrobial compounds the investigation of bioactive compounds from sixty four extracts made from biomass and the cultivation medium of sixteen Vietnamese cyanobacteria led to considerable prospect of pharmaceutical resource. The isolation of Fischerella musicolla ethyl acetate extract led to 3 antibiotic pure compounds. Structure elucidation based on MS/NMR was proposed them as known antibiotic alkaloid compounds: Hapalindolinone A and 12-Epi-hapalindole E. Five new bioactive compounds (carbamidocyclophan A, B, C, D, E) were isolated from the terrestrial Vietnamese cyanobacterium CAVN 10. These substance have antibiotic activity against Staphylococcus aureus ATCC 27853 Bacillus subtilis ATCC 6051 and are cytotoxic against MCF7 (breast cancer cell line), 5637 (bladder cancer cell line) and FL cell line. The MIC of carbamidocyclophane A, B, C has been estimated as 81, 31 and 42µg/ml. The isolated compounds were also tested for cytotoxicity against tumor cell lines, such as MCF7 (breast cancer cell line) and 5637 (bladder cancer cell line). The IC50 was estimate as 0.7 -3.3µg/ml on MCL7 cells and as 2.6 -4.7µg/ml on FL cells. These results indicate that the new compounds seem to act more cytotoxic than antibiotic to cells.

P24

Ressourcenzentrum Marine Organismen

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Marine organisms became increasingly scientific important during the last decades. They were found to be a promising source for new biotechnological interesting substances.
The screening of marine natural products has yielded a considerable number of drug candidates. Beside the human and veterinary medical implementation, natural products also are applied more frequently in food industry, cosmetics, agriculture or fish industry.
The „Ressourcenzentrum Marine Organismen" is a young biotechnological start up company. It will be outsourced from the Institute of Pharmacy and the Institute of Microbiology, Ernst-Moritz-Arndt-Universitiy of Greifswald, Germany. The company hosts an extensive culture collection of marine organisms, especially cyanobacteria, algae and fungi isolated from worldwide marine habitats. The involved biologists have a longtime experience in isolation, taxonomy, cultivation and fermentation of marine organisms as well as extraction and screening with various testsystems. In collaboration with a resident company (Baltic Analytics) we will be able to offer analysis of complex natural mixtures and structure elucidation of interesting substances.
As a private and independent company, „Ressourcenzentrum Marine Organismen" will provide marine microorganisms research services to companies and institutions involved in the discovery of new pharmaceuticals and other biotechnological procedures.

P25

Marine Fungi of Egypt: Occurrence and Biological Activity

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The marine environment is a potent source for the development of new drugs from bioactive substances, isolated from their sea inhabitants. There are three huge advantages of investigating into micro organisms: They are more effective than land-based bacteria, plants and animals, they are also cultivable in larger amounts and therefore facilitate the sustainability of marine natural substances without damage of the natural resources and – because of the difficult accessibility of their special habitat – they are not well analyzed.
In a cooperation project our groups are focussing on marine fungi in Egypt and their possible input in drug development.
For this aim we have collected mangrove- and drift-wood with colonized fungi at different time intervals in different regions along the Red and Mediterranean Sea, isolated and cultivated them and investigated in the biological activities of their extracts and purified components, respectively.
Interestingly we could find a quite different spectrum of species in summer and autumn.

P26

Studies on bacterial isolates from Lake Baikal with special emphasis on their biotechnological significance

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The industrial application of enzymes that are functional under harsh conditions has greatly increased over the past few decades. In this investigation, bacterial strains, isolated from a hot spring of the Lake Baikal region (Russia) were assessed for their diversity and further explored for their biotechnological significance. Ten aerobic bacterial isolates were obtained from the spring, characterized by slightly alkaline water with a silicate concentration of 25 µM and an outlet temperature of 55°C. It was observed that under laboratory conditions, these isolates tolerate 4.0 to 12.0 pH and the temperature between 37–55°C. These isolates were found to be able to utilize olive oil and a wide range of aliphatic and aromatic hydrocarbons. Interestingly, strain A1.37 showed an accelerated growth in minimal medium containing toxic compounds for instance adiponitrile or 1,4-Dicyano-2-butene as a sole carbon source, which provides the proof for nitrilase activity. Out of ten isolates, the cell-free culture supernatant of one strain showed lipase activity, one strain showed cellulase activity and three other strains were secreting amylases. In four bacterial isolates extracellular serine protease activity was detected by zymography with tripeptide derivatives of rhodamine 110 as the substrate. Phylogenetic analysis carried out with the use of 16S rRNA gene sequencing showed the presence of gram-positive isolates belonged to the genera Bacillus, Exiguobacteria and Kocuria as well as gram-negative isolates belonged to the genera Pseudomonas, Achromobacter and Proteobacteria. In summary, the bacterial isolates from the Lake Baikal were found to have extreme growth patterns, which highlight the importance of these strains in the exploration of valuable biotechnology products with industrial applications.

P27

A microbial key organism catalyzing autotrophic nitrate reduction in a pelagic redoxcline of the central Baltic Sea is subject to genomics

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High denitrification rates can be measured in the water column of the Gotland Deep (central Baltic Sea) at the oxic-anoxic interface when nitrate and sulfide overlap. Brettar and Rheinheimer (MEPS, 1991) postulated already earlier that chemolithoautotrophic oxidation of sulfur compounds coupled to nitrate reduction should play an important role in these redoxclines. Consequently, an Epsilonproteobacterium related to Thiomicrospira ("Sulfurimonas") denitrificans (named Helicobacteraceae G138eps1) was identified as a major key species for chemolithoautotrophic sulfide oxidation and nitrate reduction. Our aim was to determine its abundance and vertical distribution in the water column as well as to obtain a cultured isolate for autecological studies in combination with genomic and proteomic analyses. For these purposes we firstly constructed a bacterial 16S rDNA clone library for the redoxcline, screened it by RFLP and sequenced representative clones. It turned out that the most abundant clones belonged to the uncultured Helicobacteraceae. A specific 16S rRNA targeting oligonucleotide probe was designed against these nearly identical 16S rDNA sequences and used for enumeration by CARD-FISH. It could be demonstrated that this organism was quantitatively important in the redoxcline, reaching maximal cell numbers (9-12 % of total bacteria) in the nitrate/sulfide interface. However, it occurred also above the redoxcline and throughout the whole sulfidic zone. A similar result was obtained by strain specific quantitative PCR (qPCR); however, the absolute 16S rRNA gene numbers per cell were significantly highest in the area of nitrate/sulfide overlap indicating that this strain was most active in this zone. Moreover, comparing 16S rRNA based analyses of redoxclines of the central Baltic Sea, Black Sea and Cariaco Basin, it is evident that at these zones again chemolithoautotrophic sulphur oxidizing Epsilonproteobacteria belonging to the Sulfurimonas autotrophica/T. denitrificans cluster are important members of their respective microbial assemblages. However, as there occur no constant overlapping gradients of nitrate and sulfide, the dominance and wide vertical distribution of these bacteria might also depend on other combinations of electron donors and acceptors. We were able to isolate a respective strain from the Gotland Deep (called GD1), growing autotrophically on an artificial medium with thiosulfate and nitrate. Supported by the Gordon & Betty Moore Foundation, genome sequencing of strain GD1 is in progress and is expected to reveal further insights into the physiology of this key organism for pelagic redoxclines.

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Worth seeing in Greifswald

1. Pommersches Landesmuseum

Rakower Str. 9
17489 Greifswald
http://www.pommersches-landesmuseum.de

Picture gallery (Gemäldegalerie)

The thoroughly renovated classicistic building used to be a monastery in medieval times. Today it houses art work of Frans Hals, Max Liebermann, Max Pechstein, Vincent van Gogh, the famous romanticists Philipp Otto Runge and Caspar David Friedrich (who was born in Greifswald), and many others.

Geology (Erdgeschichte)

The old monasterial cellar offers an informative and entertaining walk along earth history: Spanning millions of years from the continental drift over the age of dinosaurs to the ice age, the exhibition leads from prehistoric oceans to today's coast line, from chalk to amber.

Local history of Pomerania (Landesgeschichte)

Covering 12.000 years of regional history, this part of the museum introduces the cultural background of the Pomerania area. Culture, life, landscape, politics and regional specificities of the people living on the southern Baltic shore become visible – from the first human settlements until the dawn of the Thirty Years' War.

Opening hours: Tuesday - Sunday 10.00 a.m. to 06.00 p.m.

Admission	Permanent exhibition	Special exhibition	Combined ticket
Regular fare:	4.50 €	2.00 €	5.50 €
Reduced fare:	2.50 €	1.50 €	3.00 €
Family ticket:	7.00 €	4.00 €	9.00 €
Group ticket/per person (15-25 people):	3.00 €	2.00 €	5.00 €

2. St Nicholas' Cathedral (Dom St. Nicolai)

St Nicholas'
Domplatz
17489 Greifswald
http://www.dom-greifswald.de

Greifswald's most characteristic landmark and the highest building in the city is St Nicholas' Cathedral. The medieval gothic brick church is one of North Germany's most impressive sacral buildings and well worth a visit. The ascent to the observation deck on top of the cathedral offers a splendid view of Greifswald and its surroundings as far as the island of Rügen and is highly recommended.

Opening hours: Monday to Saturday 10.00 a.m. to 04.00 p.m.
Sunday 12.00 a.m. to 03.00 p.m.

Ascents to the tower can be started until 30 minutes before closing, admission is 1.50 € (regular) / 1.00 € (reduced fare).

3. Caspar David Friedrich's birthplace (Friedrichsche Seifensiederei)

Caspar-David-Friedrich-Zentrum
Lange-Straße 57
17489 Greifswald

The well known romanticist Caspar David Friedrich, who was born here in 1774, is regarded Greifswald's most famous son. Today, his family's place, a former soap manufactory, houses the Caspar-David-Friedrich-Centre. The comprehensive multimedia-based exhibition on the ground floor mainly focuses on the life and art work of C. D. Friedrich. In the basement rooms, the original appearance of which was thoroughly restored, the historical requisites of soap and candle production in the house are on display. The centre features a small library with selected literature about the artist and a museum shop that offers unique art products as well as hand made candles and soap.

Opening hours: Tuesday to Friday 10.00 a.m. to 06.00 p.m.
Saturday and Sunday 11.00 a.m. to 05.00 p.m.

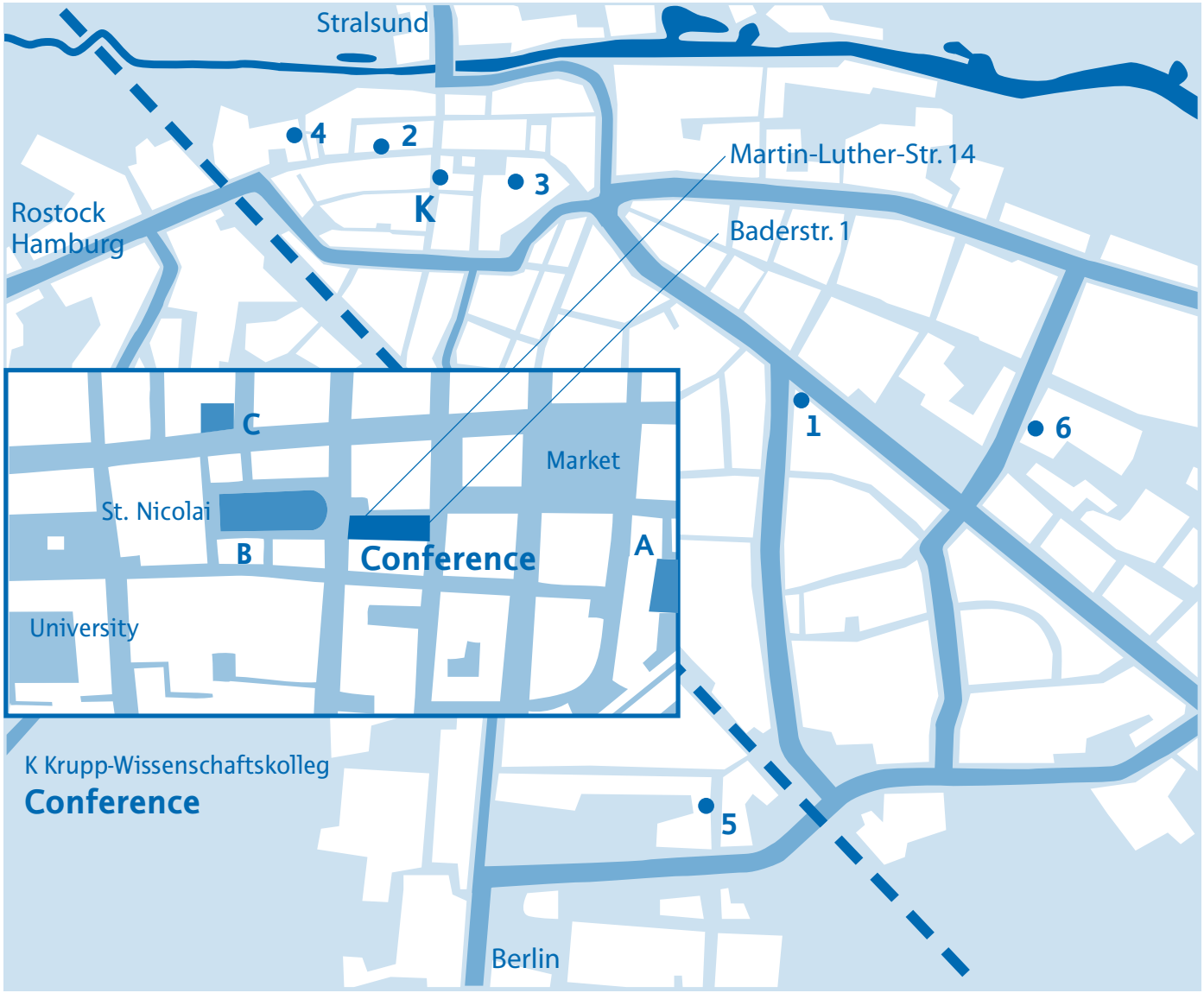
For additional information and more recommendations as to what is worth seeing in Greifswald visit the official Greifswald homepage http://www.greifswald.de.
The monastery ruins of Eldena, the fishing village of Greifswald-Wieck and a variety of historical buildings in Greifswald's old town are introduced here.

The tourist information in the town centre offers personal help to your questions as well:

Greifswald-Information
Rathausarkaden
Am Markt
17489 Greifswald

Opening hours: Monday to Friday 09.00 a.m. to 06.00 p.m.
Saturday 10.00 a.m. to 02.00 p.m.

MAP



1 Hotel Best Western
2 Hotel Kronprinz
3 Hôtel Galerie
4 Hotel Adler
5 Mercure Hotel Am Gorzberg
6 Parkhotel

A Pommersches Landesmuseum
B St. Nicolai
C Friedrichsche Seifensiederei