Marine
Microbial Biodiversity,
Bioinformatics & Biotechnology

Grant agreement n°287589

Acronym: Micro B3

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Deliverable 9.5

Report on all KT and TT Workshops Held until Mid-Term

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Summary

This report contains an overview about all Knowledge and Technology Transfer (KT and TT) workshops planned and details on two workshops held until the mid-term of the Micro B3 project.

The first Micro B3 Stakeholder Workshop in WP 9 had a strong legal focus and was held under the lead of UCL with input from the full legal team of WP 8. It took place in Brussels on 27 and 28 February 2013 with 37 participants. The full title of the workshop was "Towards a Model Agreement on Access and Benefit Sharing (ABS) for Marine Genetic Resources (with a focus on marine micro-organisms) - proposed best practices to access MGRs and support metagenomic science for utilization in data-driven global research collaborations based on the Convention on Biological Diversity, taking into account the Nagoya Protocol".

Six providing countries participated in the workshop with representatives from scientific, legal and ministerial sectors (Algeria, China, Costa Rica, Egypt, Kenya, Turkey). Ten Micro B3 partners and two representatives of three other EU consortia from marine KBBE projects (BlueGenics, PharmaSea, SeaBioTech) were also attending as well as one representative of the research infrastructure on microbial collections, MIRRI.

On the first day legal and scientific experts from Micro B3 and invited speakers introduced the framework of the ABS agreement and its synergies with research and development. On the second day guest speakers presented two practical case studies. Then stakeholders analyzed and discussed the core clauses of the model Access and Benefit Sharing Agreement for Marine Genetic Resources in two parallel sessions: one group dealing with access to genetic material and the other with data management. Their input was integrated into the development of an improved Micro B3 Model Agreement.

The first Industrial Expert Workshop, titled "Harvesting Environmental Genomes for the Development of Biocatalysts", was organized at the University Groningen, on 14 and 15 October 2013. It was a joint endeavour of the Micro B3 and MetaExplore FP7-supported projects. A total of 72 participants from biotechnological industry and academia from 16 different European countries met for this two-day workshop. Four lecture sessions were held and one final panel discussion as a more interactive element, all striking a balance between academic and industry input. The experts exchanged knowledge on current genome-based approaches for discovery of new bioactive compounds and on how new, especially bioinformatic tools can support the understanding of complex genomic and proteomic data for use in engineering of new enzymes and production of small molecules.

Several presentations emphasized the importance of powerful bioinformatics approaches and computational tools for discovery of new enzymes and for tailoring their properties for industrial applications. The strong position of European companies in industrial biocatalysis was illustrated by examples of small-scale and large-scale processes in the fields of eco-friendly production of antibiotics, renewable polymers, functionalized carbohydrates, and enantiopure building blocks for the preparation of pharmaceutical products. Representatives

of global players like DSM, BASF, Sigma-Aldrich and Corbion demonstrated that they are at the forefront of introducing such new bio-based technologies.

The aim of a final panel discussion was to allow representatives from academia, small and medium enterprises (SME) and large companies from the biotechnology sector to share their perspectives on the challenges and needs in using bioinformatics services and tools for biotechnology and especially biocatalysis.

Three key questions were raised on:

1) Uses of bioinformatics in industry,

2) Bottlenecks in marine bioprospecting, and

3) Lessons learnt from industry-academia collaborations.

The importance of collaboration of industry and academia in biotechnological research and technological development (RTD) projects was highlighted, involving SMEs with complementary areas of expertise. The participant's contributions also reflected intensive industry-academia collaboration achieved within and beyond FP7 projects. Both large companies and SMEs participate in relevant networks and projects. The panel discussion highlighted the importance of training, in order to make SMEs more competitive, and to train bioinformaticians in customer-oriented approaches.

An open discussion about the role of public databases and the optimization of data sharing demonstrated the importance of genomic databases to include a set of standardized contextual data to predict (novel) functions to be linked to enzyme properties. International and open database(s) describing expressed enzyme properties appear of utmost importance for full exploitation of metagenomic sequence data.

Table of Content

General Introduction

In WP 9 overall, a threefold approach is being implemented in addition to an informative website, factsheets, newsletters and other contributions to dissemination (film, sessions at conferences).

1. **Two stakeholder workshops** for the interested public, including policy-makers, to inform them about marine microbial diversity, the genomic revolution, its application options and aspects of sustainable use of marine genetic resources. One was held in February 2013 and is reported on in this deliverable;
2. **Three industrial expert workshops** for promoting bioinformatics tools and other Micro B3 results and increase interaction based on Knowledge and Technology Transfer (KT and TT) with industry. One was held in October 2013 and is reported on in this deliverable;
3. **Scientific training,** which is not part of this deliverable report, is organized into a training pipeline, including several short training courses and one summer school. Two courses have been held and are reported on in the Deliverable D9.6 “Report on training pipeline and courses held until mid-term”.

Besides an ambitious training programme for scientists, two series of workshops are thus planned to establish a baseline and make project results accessible for researchers, industry, the public and policy makers. These workshops are reported on in this deliverable, with a focus on one stakeholder and one industrial expert workshop held in 2013.

Overview on all Stakeholder Workshops

For the two stakeholder workshops, topics were derived from the Training and Information Needs Survey (the questionnaire is reported in Deliverable 9.1 and the complete survey is reported on in Deliverable 9.22). In the Description of Work topics like Intellectual Property Rights (IPR), scientific potential, biotech options, environmental impact were planned. The topics should inform stakeholders from regional, national and international policy-making bodies and application and protection options for marine microbial diversity discussed, e.g. in the context of implementation of the Convention on Biological Diversity (CBD), especially the Access and Benefit Sharing aspects (ABS). This plan was later refined with feedback obtained from the IPR-part of the Needs Survey (Figure 1), as already explained in D9.22 and detailed in the text and table below.

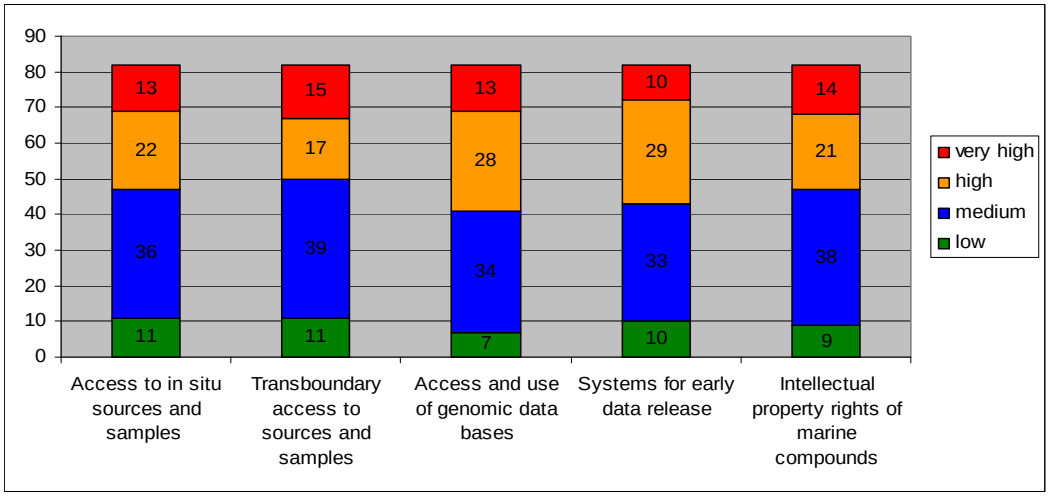


Figure 1: Results of the needs survey on topics related to legal and governance issues

The first stakeholder workshop had a strong legal focus, as this was of best use to the Micro B3 project work ongoing within WP 8 on drafting a model agreement. It was lead by UCL with input from the full legal team of WP 8. It has been held in Brussels on the 27 and 28 February 2013. The full title of the workshop was "Towards a Model Agreement on Access and Benefit Sharing for Marine Genetic Resources (with a focus on marine micro-organisms) - proposed best practices to access MGRs and support metagenomic science for utilization in data-driven global research collaborations based on the Convention on Biological Diversity, taking into account the Nagoya Protocol".

The second stakeholder workshop will focus on areas of specific interest to Mediterranean stakeholders and will be led by CIESM. It is planned for the first half of 2015. It shall present outcomes of Micro B3 to target groups representing governmental and scientific involvement in environmental and biotech research infrastructures in the Mediterranean Basin. Micro B3 partners will report on progress in bioinformatics for better understanding and monitoring the marine environment, demonstrate potential of data for marine biotech development and explain the need for uniform standards and IPR rules. These will include transboundary access issue and data policies and the CIESM Code of Conduct. One goal is to further capacity development through informing national strategies on research infrastructure planning and to discuss options for future collaborations.

Table 1: Schedule of stakeholder workshops

Title of the Stakeholder Workshop	**Venue, attendees**	**WP9 team-leader**	**Date, duration**
"Towards a Model Agreement on Access and Benefit Sharing for Marine Genetic Resources"	Brussels, 37 persons	**UCL**, EMPA, CIESM	27-28 February 2013
Informing on Micro B3 outcomes linked to research infrastructures	Mediterranean location, approx. 20 persons	**CIESM**, Jacobs Uni, UCL, EMPA	Month 36-42, 2 days maximum

Overview on all Industrial Expert Workshops

Intensive exchange of information is planned with industrial partners, especially from SME within the white or industrial biotechnology sector, to discuss their needs and impart knowledge on the exciting areas of marine (meta)genomics, transcriptomics and proteomics for use in biotechnology. In this context Micro B3 partners have already been providing input (presentations, panels, think tanks) in conjunction with industry events like BioMarine, EFIB, Industrial Biotechnology workshops and other events which are attended by the targeted industrial sectors.

In addition to this, three Industry Expert Workshops (Table 2) are planned by the Micro B3 project in order to involve industry, especially SMEs, in Knowledge and Technology Transfer (KT and TT). These short workshops with small targeted participant groups are designed to enable intense discussions and open exchange of ideas.

Topics chosen for the workshops are planned based on the Needs Survey and include the development and use of standards, databases and further applied features of Micro B3. Presentations cover screening results, evaluation of IPR possibilities, bioinformatic tools and exchange of protocols (see Figure 2).

An effort was made in the last months of 2012 to obtain more industry feedback on their topics of interest, as reported in the Deliverable D9.1 “Stakeholder analysis”. Every workshop is envisaged to include bioinformatics topics and at least one talk on IPR, as shown in the figures below, targeted towards the industry sector addressed.

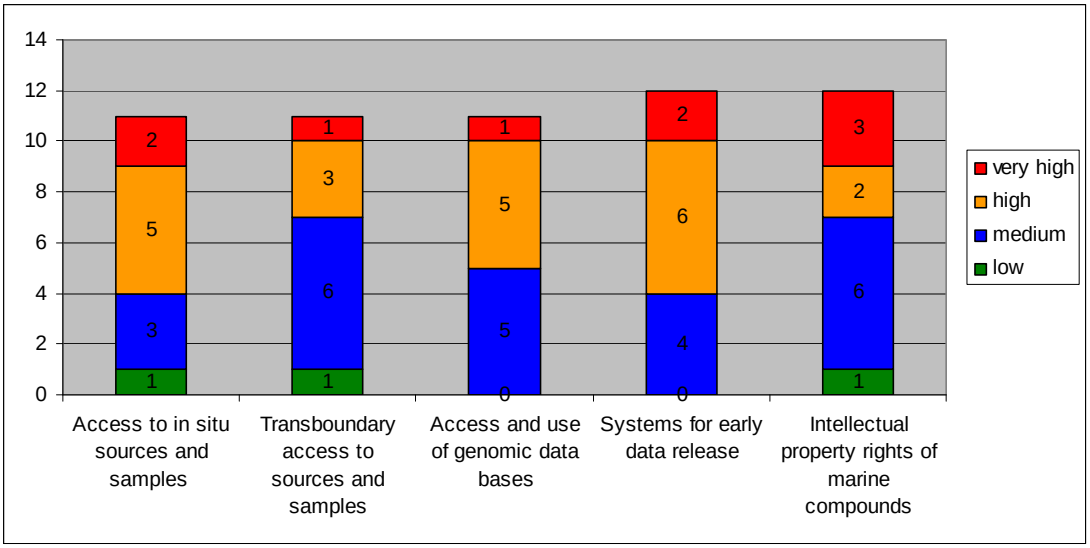


Figure 2: Interest by industry in IPR and legal issues according to the Nagoya Protocol to the CBD & UNCLOS (only 16 detailed replies from industry were obtained overall)

The following three bioinformatic topics are of industry interest (Figure 3):

- Opportunities and pitfalls of new bioinformatics tools for marine genomics
- Opportunities and pitfalls of new sequencing technologies
- Contextual data for exploration/exploitation of marine resources.

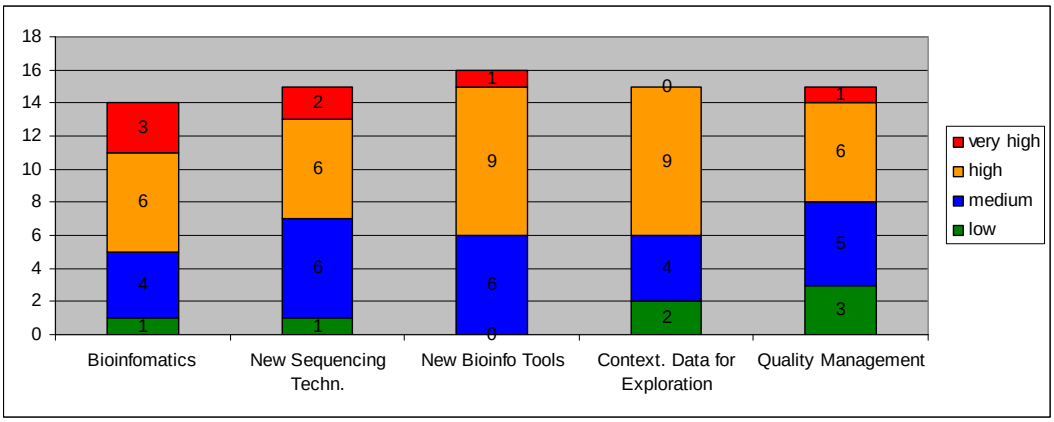


Figure 3: Results of the needs survey on topics of interest to industry related to bioinformatics

From a further set of questions related to marine biotechnology, the following three out of five proposed topics were of higher interest to industry:

- Genomic approaches for exploiting marine-derived compounds
- Green biotechnology (food, feed, fibre, fuel) meets blue biotechnology
- New hosts for screening of bioactive compounds.

EMPA and the lead parties for the workshops are building on this information to develop the formats and contents of three expert workshops in conjunction with Micro B3 results of interest to industry stakeholders. In each workshop basic and applied scientists involved in Micro B3 will present approaches and findings, and speakers from industry will elaborate on their application areas. Foci will be different, ranging from industrial biotech and applied catalysis to what bioinformatics has to offer for bio-actives, green and blue biotech (see Table 2). Finally the added value of the Micro B3 Information System will be presented to industry, integrating contextual data to interpret the high amount of marine genomic information (big data) based on next generation sequencing (NGS) technologies.

The first Industrial Expert Workshop, titled “Harvesting Environmental Genomes for the Development of Biocatalysts”, was organized at the University Groningen, on the 14 and 15 October 2013 and is reported on in detail below.

The second workshop is planned in Madrid in November 2014 under the lead of the large industrial partner PharmaMar together with the Spanish SME partner Bio-Iliberis, both active in marine biotechnology. This workshop will promote several topics especially to industry representatives from Mediterranean countries. It will address cultivation/new hosts for screening, bioinformatics tools to enhance uses of metagenomes for industry, IPR/ABS aspects of marine-sourced compounds, etc., with a focus on blue and environmental biotechnology and on sustainable use of marine microbial diversity.

The topics for the last workshop to be held in 2015 are not fully decided upon. They will include the development and use of standards, databases and further applied features of Micro B3 bioinformatics work. New bioinformatics tools addressing opportunities and pitfalls of constantly evolving new sequencing technologies will be presented. The workshop could

also promote the IPR model agreements finished by then, with a view to commercial applications.

This last industry workshop will probably also focus on the outcomes of WP 7, which may include new hosts for screening of bioactive compounds and/or novel functions. Details will be decided in conjunction with the final conference sessions to maximise targeted dissemination of our findings and results.

Table 2: Schedule of industrial expert workshops

Title of industrial workshop	**Venue, attendees**	**WP9 team-Leader**	**Date, duration**
Harvesting Environmental Genomes for the Development of Biocatalysts	Groningen, 72 persons	**UGRO**, EMPA (joint workshop with MetaExplore project)	14-15 October 2013
Marine Micr'Omics for Biotech Applications	Madrid, up to 60 persons	**PharmaMar, BIO-ILIBERIS**, CIESM, EMPA	3-4 November 2014, (calendar week 46)
Bioinformatics Tools and Biotech Results of Micro B3	To be announced	RIBOCON, Interworks, MPI, EMPA	2015, 1-2 days

First Stakeholder Workshop

The first stakeholder workshop had a strong legal focus and was lead by partner UCL and co-organized by EMPA with input from CIESM and the full legal team of work package 8 (WP 8). It took place in Brussels on the 27 and 28 February 2013. The full title of the workshop was

> **Towards a Model Agreement on Access and Benefit Sharing for Marine Genetic Resources (with a focus on marine micro-organisms)**
>
> **- Proposed best practices to access MGRs and support metagenomic science for utilization in data-driven global research collaborations based on the Convention on Biological Diversity, taking into account the Nagoya Protocol -**

The workshop aimed at getting feedback on the first draft of Micro B3 Access and Benefit Sharing Model Agreement from various stakeholders: legal experts, national authorities dealing with access to genetic resources, scientists, industry representatives and other EU project partners; in order to improve the draft. Many comments were obtained and put to use in adapting the model agreement. As the "Micro 3 Model Agreement on Access to Marine Micro-Organisms and Benefit Sharing" is now available from the website, details of this process are not reported upon in this deliverable.

(www.microb3.eu/sites/default/files/pdf/MICRO_B3_ABS_model_agreement_17122013 explanatory notes.pdf)

Planning Phase

In the context of WP 8 dealing with Intellectual Property Rights and ABS management for marine research and bio-prospecting), a Model Agreement on Access to and Benefit Sharing of Marine Micro-Organisms (as a special case of marine genetic resources, MGR) has been drafted. The Model Agreement aims to deliver a set of contractual tools that can be used by major European initiatives in this field.

In order to receive targeted stakeholder input into the development of this agreement it was decided to dedicate the first stakeholder workshop of WP 9 to the topic of marine genetic resources (MGR). The aim of the workshop was to present the draft model agreement, and to further discuss the agreement and its core clauses with the main stakeholders involved in research and bio-prospecting of MGR from:

- provider and user countries' authorities (issuing permits)
- the scientific community on the provider and user side
- industry actors
- legal experts, and
- representatives from databases and culture collections.

It was decided to keep the number of attendees limited and upon invitation only. In this context, a list of experts has been carefully agreed on with inputs from all WP 8 partners, to ensure the attendance of representatives from different communities and geographical areas. The agenda and list of invited speakers was agreed after several rounds of thorough consultation among all WP 8 members. The presentations were chosen to enable a similar

level of understanding of actual issues on marine genetic resources needed to understand and provide insight into the legal work undertaken by WP 8 so far.

It was agreed to organize the workshop with a focus on lectures on the first day, concluding with a final panel discussion focussing on industry needs and interests; and a more interactive second day for stakeholders to analyse the core clauses of the agreement in two parallel working groups.

CIESM and EMPA Bremen helped to establish contact to stakeholders from the MED area and from industry, involved in marine research and marine genetic resources. Uni Leuven, as the lead party for this workshop, contacted all pre-selected participants and invited speakers, informing them about the issues and the input expected from them, as well as organizing the venue and preparing the workshop documents. Uni Leuven was also responsible for dealing with extra costs based on a dedicated workshop budget. All EU project participants did cover travel and accommodation costs from their own budgets.

To increase visibility for outreach reasons, the workshop was announced on the MicroB3 website under the events section:

www.microb3.eu/events/meetings/micro-b3-wp8-stakeholder-workshop-brussels

Implementation Phase

On the first day legal and scientific experts from Micro B3 and invited speakers introduced the framework of the ABS agreement and its synergies with research and development. On the second day two practical case studies were presented by two guest speakers. Then stakeholders analyzed the core clauses of the agreement in two parallel sessions: one group dealing with access to genetic material and the other with data management. The focus was put on the model Access and Benefit Sharing Agreement for marine genetic resources, under development as a deliverable of WP 8.

Six providing countries participated in the meeting with representatives from scientific, legal and ministerial sectors (Algeria, China, Costa Rica, Egypt, Kenya, Turkey). Ten Micro B3 partners and at least two representatives of three other EU consortia from marine KBBE projects (BlueGenics, PharmaSea, SeaBioTech) were also attending as well as one representative of the research infrastructure on microbial collections, MIRRI.

The Workshop: Key Points Presented and Discussed

The thematic sections of the workshop dealt with:

- The legal background for the MICRO B3 Model ABS Agreement
- The scientific and bioinformatic background for the MICRO B3 Model ABS Agreement
- Introduction to the analysis and discussion of the MICRO B3 Model ABS Agreement

- Experiences with Access to (Marine) Genetic Resource for commercial research and development: Industry panel discussion with representatives from BlueGenics, PharmaSea, SeaBioTech; moderated by Dr. Johanna B. Wesnigk, EMPA Bremen.
- Practical case studies:
    - Accessing geothermal microbes in Iceland
    - ABS of Marine Genetic Resources in the Antarctic Treaty System
- Analysis and discussion of the MICRO B3 Model ABS Agreement:
    - PANEL A: Access to and utilization of genetic resources
    - PANEL B: Transfer of genetic resources and data management.

The model agreement was discussed and improved as the different participants gave their inputs. The providing countries specified their needs and explained difficulties to give access to their resources, according to their national policies and political positions. The scientists highlighted the need to be informed on the ABS steps and procedures to be followed when organizing sampling. They also remarked on the need for the Model Agreement to be anchored in scientific practices. They were very helpful in adjusting articles which are especially relevant for performing their scientific activities. As a side topic the Ocean Sampling Day initiative was also discussed and the involvement of four possible new sites for sampling was considered.

During the panel discussion industry and academic members highlighted their positions in relation to applied research and issues related to marine and microbial aspects of intellectual property rights. They freely shared experiences in dealing with access to marine genetic resources and asked for improved clarity or rules and regulations in order to reach legal certainty. In this context they demanded a precise list of ABS steps to be followed in order to facilitate respecting the Convention on Biological Diversity and its Nagoya Protocol, which is not yet in force.

The information which was distributed to the attendees can be found in the Annex 1 to this report. It includes the agenda and the participants list. Instead of the draft model agreement discussed then, a link to the actual version of the Model Agreement (as promoted by Micro B3 on the web portal) is provided below:

www.microb3.eu/sites/default/files/pdf/MICRO_B3_ABS_model_agreement_17122013_explanatory_notes.pdf

First Industrial Expert Workshop

According to a proposal by the University of Groningen, who accepted to lead on this, the first Industrial Expert Workshop has been organized in collaboration with the coordinator of the MetaExplore project. It was titled "Harvesting Environmental Genomes for the Development of Biocatalysts", and was in the cultural centre Oosterpoort, Groningen on the 14 and 15 of October 2013. It included 17 invited speakers from academia and industry and was divided into four sessions and a final panel discussion. 17 Posters were shown during the workshop.

A total of 72 participants from industry and academia from 16 different European countries met for this two-day workshop. They exchanged knowledge on the current approaches on the discovery of new bioactive compounds and how new bioinformatics tools can support the understanding of complex genomic and proteomic data for use in engineering of new enzymes and production of small molecules.

The programme, abstracts of all talks, the list of participants and other information are available in the Annex 2 of this report. The following report covers all the stages of preparation and implementation in detail. This is done in order to facilitate the organisation of coming Micro B3 workshops in a similar way, which are planned by different lead partners and in different locations.

Planning Phase

In April 2013 members of the Organization Committee met in Groningen. Before moving forward to develop the agenda, participants took time to agree on the objectives of this workshop and to examine how to link with other EU projects to create a high level of participation of selected target groups.

The Organization Committee was composed of:

Dick Janssen, Univ. of Groningen (chair)
Jan-Dirk van Elsas, Univ. of Groningen (MetaExplore coordinator)
Johanna Wesnigk, EMPA, Bremen (Micro B3)
Rita Dunker, EMPA Bremen (Micro B3)
Sandra Haan and Tamara Hummel, Univ. Of Groningen (local organization)

Scientific Advisors:

Frank-Oliver Glöckner, Jacobs University (Micro B3 coordinator)
Dietmar Pieper, HZI, Braunschweig (MagicPAH coordinator)
Marco Fraaije, Groningen (Oxygreen coordinator)

Development of Content

The Committee agreed on the programme, including four sessions, one panel discussion and chair persons for all of them, and on a list of invited speakers. This was revised and expanded with the help of the scientific advisors.

- Session 1: New enzyme discovery and engineering
- Session 2: Bioinformatics and discovery
- Session 3: Industrial needs
- Session 4: Databases, discovery and engineering
- Panel discussion around three key questions:
  1) use of bioinformatics in industry,
  2) bottlenecks in marine bioprospecting,
  3) lessons learnt from industry-academia collaborations.

The three project coordinators not present during the first planning meeting helped to contact high-level speakers in the fields of applied microbiology, biodegradation and biocatalysis for the sessions (Fraaje, Glöckner) and the panel (Pieper). Several drafts of the programme were circulated to committee members, chairpersons and speakers during this phase.

Organization

The Organization Committee also selected the final date, 14-15 October 2013 (which had to be changed once from September, due to the overlapping MaNaPro conference led by WP 7 partner PharmaMar) and created a timeline and a to-do list. This list included invitation details like cover letters for speakers, participants and multipliers and other practical details regarding registration fee, registration modalities, hotel options, venue in Groningen, catering, etc..

The budget was discussed and what to cover with the 10,000 Euro Micro B3 budget earmarked for each workshop. Costs for travel and accommodation of external invited speakers, for the abstract book and some costs for the external location could be covered as envisaged. All EU project partners used their own funds. As the aim for this workshop was a larger audience (60 – 80, due to several projects involved) it was decided that a small registration fee (80 Euro) was needed to cover further (external) venue costs for catering, including coffees, lunches and one workshop dinner.

During the planning phase, EMPA invited all speakers from the Micro B3 project and from most German companies, whereas Univ. Groningen focussed on the MetaExplore members as well as Dutch companies and other high-level industry contacts. In addition to this meeting, weekly email communications among the Organization Committee members took place.

Furthermore, Univ. Groningen planned and managed all local elements (venue, meals, registration of participants) as well as taking over the detailed information of speakers and the collection of abstracts of talks and posters for the abstract book to be distributed to all participants at the meeting.

Promotion Phase

During this phase, special efforts were made to announce the workshop to diverse target groups especially from industry in several countries to promote participation. This promotion of the workshop has been done through the following channels:

- EMPA provided early on a list of potentially interested attendees from companies EU-wide to Univ. Groningen for selection, which was complemented with their expertise and contacts who were invited with a personal approach.
- Univ. of Groningen invited partners from other EU projects (KyroBio, Oxygreen, MetaExpolore, P4FIFTY, ENEFP, BioNexgen), and informed the multipliers BE-Basic consortium and the IBOS network. EMPA informed many project partners of related marine projects (PharmaSea, Magic PAH, SeaBiotech).
- EMPA furthermore sent the workshop announcement to more than 50 multipliers, i.e. national and EU-wide associations and clusters who regularly produce newsletters on marine or industrial biotech issues and/or announce biotech events on their websites (for feedback see below).
- The Micro B3 Newsletter from 12 September was used to inform subscribers
- The draft final programme was published on the Micro B3 website under “events” and “workshops” from June 2013 on.
- Registration was opened in August on the Univ. of Groningen website. (http://www.microb3.eu/events/workshops/micro-b3-industry-expert-workshop and http://www.rug.nl/research/gbb/education/workshop/microb3-metaexplore/)

Assessing the feedback from multipliers, announcements for the workshop have been published as follows:

- The EU wide European Biotechnology News, which featured the workshop on its website event section but not in their biweekly newsletter
- UK-based Marine Biotechnology Newsletter (September 2013)
- Industrial Biotech Norway Network website & newsletter
- the North German Life Science Agency Norgenta, which announced the workshop on the events section on their website
- the large German industrial biotech association (CLIB), which also announced the workshop.

Implementation Phase: Key Points Presented and Discussed

Session 1: New Enzyme Discovery and Engineering

Glieder, Anton	*Novel enzymes from transcriptomes* ACIB, Austria
Kruus, Kristiina	*Discovery of novel cellulases and hemicellulases from unique metagenomic libraries* VTT, Finland
Wellington, Elizabeth	*The studying enzymes in soil using metaproteomics* University of Warwick, United Kingdom
Yakimov, Michail	*Organisms and enzymes from deep-sea hyper-saline lakes* IAMC, Italy

Key points presented by Dr. Glieder:

- Transcriptome sequencing and expression in yeasts offers new opportunities to discover novel enzymes.
- Example: PharmaPLE™ (recombinant pig liver esterase), developed by company DSM, as model for feasibility study.

Key points discussed:

- Advantages and disadvantages of using fission yeast for expressing plant genes.
- Aspects covered were the timeline compared to more classical approaches and predictability in the light of useful expression.

Key points presented by Dr. Kruus:

- A successful screening approach was shown for discovering unique cellulases and xylanases from plasmid and fosmid libraries with a focus on thermostability.
- Various novel enzymes were discovered in screens after enrichment of mostly bacterial metagenomes from several sites, including hot springs.

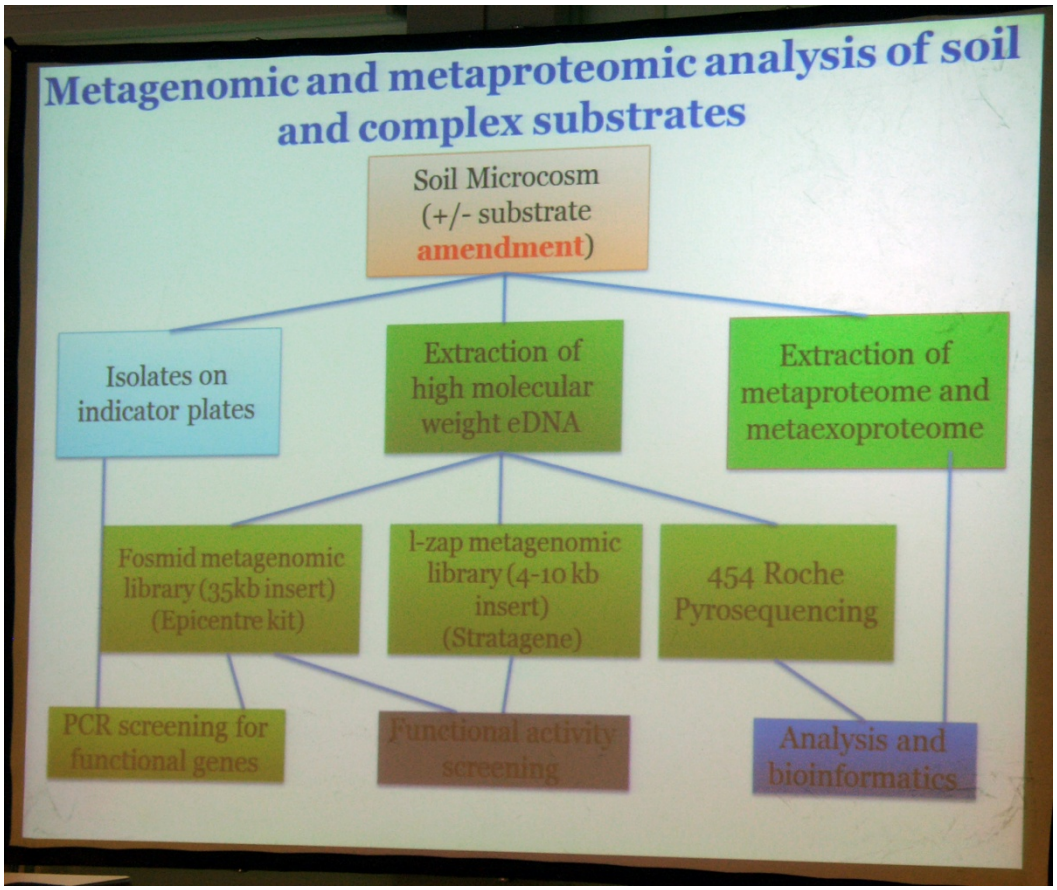
Key points discussed:

- Differences in lignocellulosic enzymes between bacterial endoglucanases and fungal exoglucanases. Expression in different bacterial hosts.
- Mechanism behind adaptation of enzymes under study to increase thermostability, due to increase of hydrophobic interactions and/or changes in covalent binding?

Key points presented by Dr. Wellington:

- The role of chitin in diverse industrial applications.

- Metaproteomic approaches of extracellular and total soil enzymes involved in the breakdown of natural polymers, like chitin and lignocellulose.
- Use of metagenomic sequence data for identification of proteins and recovery of genes.



Slide from Dr. Wellington's presentation showing metagenomic and metaproteomic approaches for analysis of extracellular and total soil enzymes based on PCR screening; functional activity screening; analysis and bioinformatics

Key points presented by Dr. Yakimov:

- New esterases were found in hypersaline anoxic marine lakes. They are promising candidates for the synthesis of optically pure biologically active substances that may be useful to provide access to pharmaceutical intermediates.
- Discovery of microbes near the phylogenetic intersection dividing the prokaryotic domains of Archaea and Bacteria.

Key points discussed:

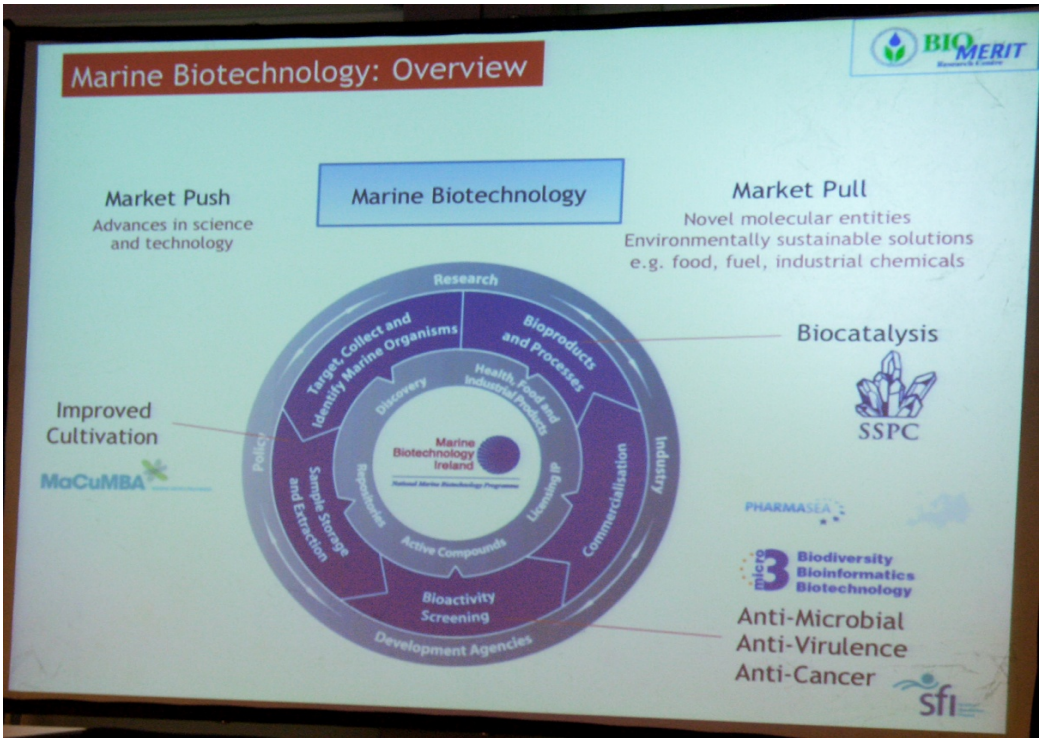
- The high microbial diversity of these poly-extreme environment (high salinity, high pressure, high magnesium concentration and anoxia) as a potential source of new enzymes.

Session 2: Bioinformatics and Discovery

Chair: Peter Golyshin, University of Bangor	
O'Gara, Fergal	*Current trends in marine biotechnology* BIOMERIT, University College Cork, Ireland
Wohlgemuth, Roland	*New biocatalysts for industrial applications* Sigma-Aldrich, Switzerland
Medema, Marnix	*Genomics-based discovery of bioactive compounds* MPI Bremen, Germany
Janssen, Dick	*Computational approaches in enzyme discovery and engineering* University of Groningen, The Netherlands

Key points presented by Dr. O'Gara:

- Overview on marine biotechnology.
- Sponges as source for marine bioactive compounds.

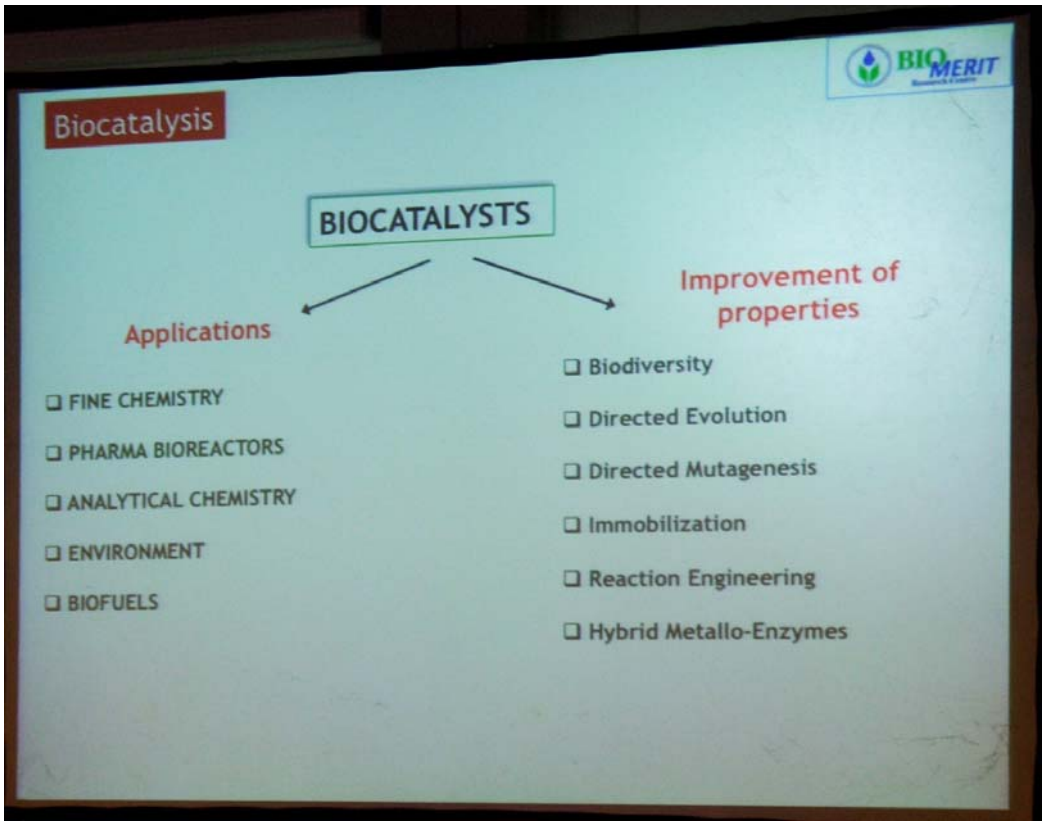


Slide from Dr. O'Gara's presentation on marine biotechnology

- Importance of the type of screening approach (classical, metagenomic, cloning, metatranscriptomics) for discovering new compounds.

Key points discussed:

- Expression of secondary metabolites.

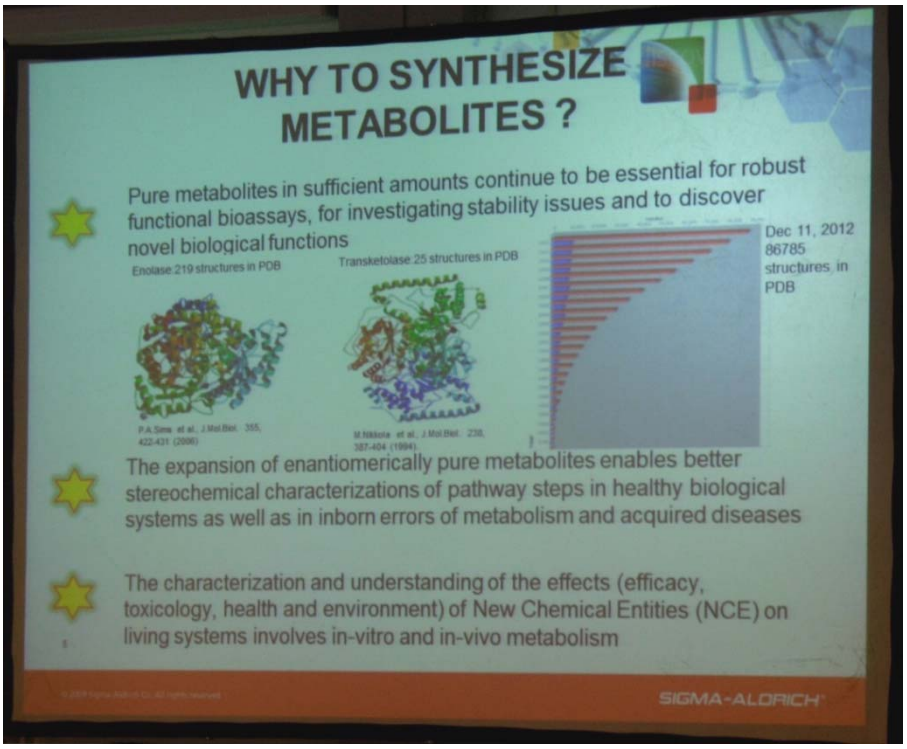


Slide from Dr. O'Gara's showing the key issues for developing better biocatalysts

Key points presented by Dr. Wohlgemuth:

- Need to find best combination of chemistry and enzymatic steps.
- Examples provided were:
    - D-glyceraldehyde 3-phosphate (for medical purpose).
    - C-C bond formation (C2-chain elongation from hydroxypyruvate).
    - Mevalonate, using asymmetric phosphorylation led to product and useful byproduct in one step with high yield.
    - Isoprenoid pathways.
- Reduction of steps, i.e. through de(oxy)dehydratases from *Th. proteus*.
- Using intense chirality as advantage.
- Selective defunctionality of carbohydrates.

Dr. Wohlgemuth presenting metabolic pathway map for targeting key steps



Dr. Wohlgemuth's slide substantiating the "Molecular Economy of Biocatalysis"

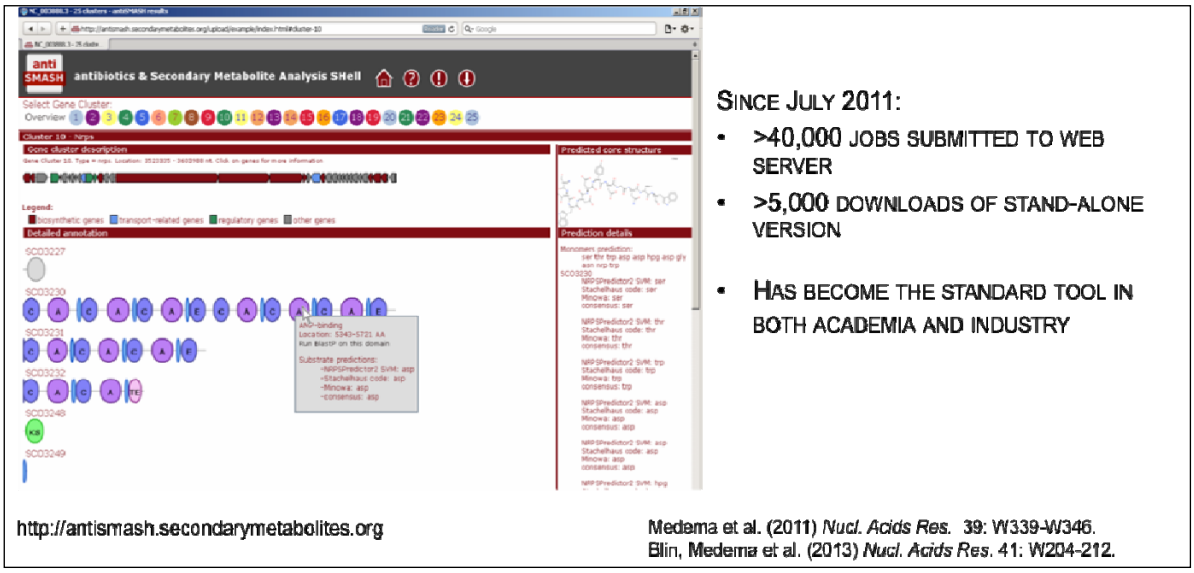
Key points discussed:

- Chemical catalysis is increasingly being replaced by and/or combined with biocatalysts.
- Reaction engineering is needed for handling labile intermediates.

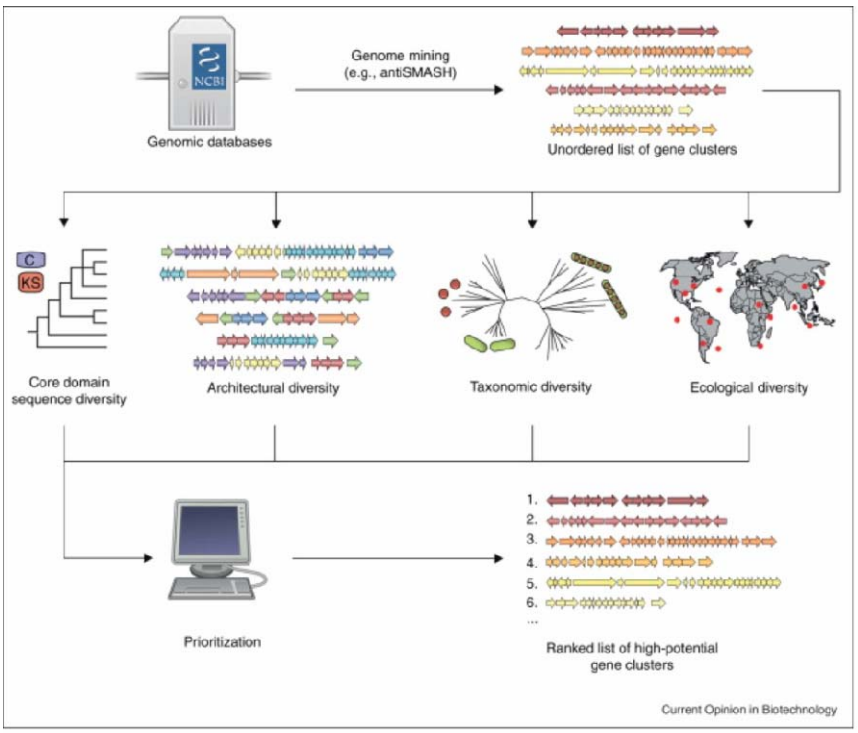
Key points presented by Dr. Medema:

- An innovative computational pipeline was developed with the AntiSmash platform (http://antismash.secondarymetabolites.org) for antibiotics and secondary metabolites analysis and chemical structure prediction.

- Its usefulness for evolutionary distance analysis was demonstrated.
- Quantitative and comparative analysis of biosynthetic gene cluster can be performed on microbes on a global scale.



Overview on antiSMASH: A Web Server for the detection and analysis of biosynthetic gene clusters (Dr. Medema, MPI)



Frasch, Medema et al. (2013) *Curr. Opin. Biotechnol.* doi:pii: S0958-1669(13)00063-3

Slide from Dr. Medema's presentation on mapping and prioritizing gene cluster diversity at various levels

Key points discussed:

- How to link these gene clusters with ecological information.

- Data storage, i.e. query information is not stored, as IP sensitive.
- Most data sets are from cultivated organisms. Aim validated standard data set.

Key points presented by Dr. Janssen:

- Development of the FRESCO framework for enzyme stabilisation. This computational approach generates small libraries which large numbers of thermostability-enhancing mutations. Combining confirmed mutations provides highly thermostable enzymes
- The use of docking and molecular dynamics simulations for predicting the potential of (marine) gene sequences for specific biocatalytic conversions, which wikll reduce the amount of required experimental screening.

Key points discussed:

- To what extent can computational prediction be used to focus experimental work?
- Is a crystal structure always required for computational predictions?

Session 3: Industrial Needs

Chair: Dick Janssen, University of Groningen	
Baldenius, Kai	*Industrial biocatalysis- enzyme catalysis for efficient chemical production* BASF, Germany
van der Laan, Jan-Metske	*Enzyme discovery for biocatalysis and synthetic biology* DSM Biotechnology Center, The Netherlands
Ruijssenaars, Harald	*Bioprospecting – an industrial perspective* Purac/Corbion, The Netherlands
Leggewie, Christian	*Improvement of biocatalysts for industrial applications* evocatal, Germany

Key points presented by Dr. Baldenius:

- An overview was provided of BASF interest in using enzymes for chemical production, starting with chiral intermediates for pharmacy (Chipros using lipases for amine acetylation), now extended to 200 major basic products.
- Examples provided were
    - Acrylamide via nitrile hydratase;
    - Polyester and polyamides from natural oils, e.g. via oleate hydratases and sebacic acid.

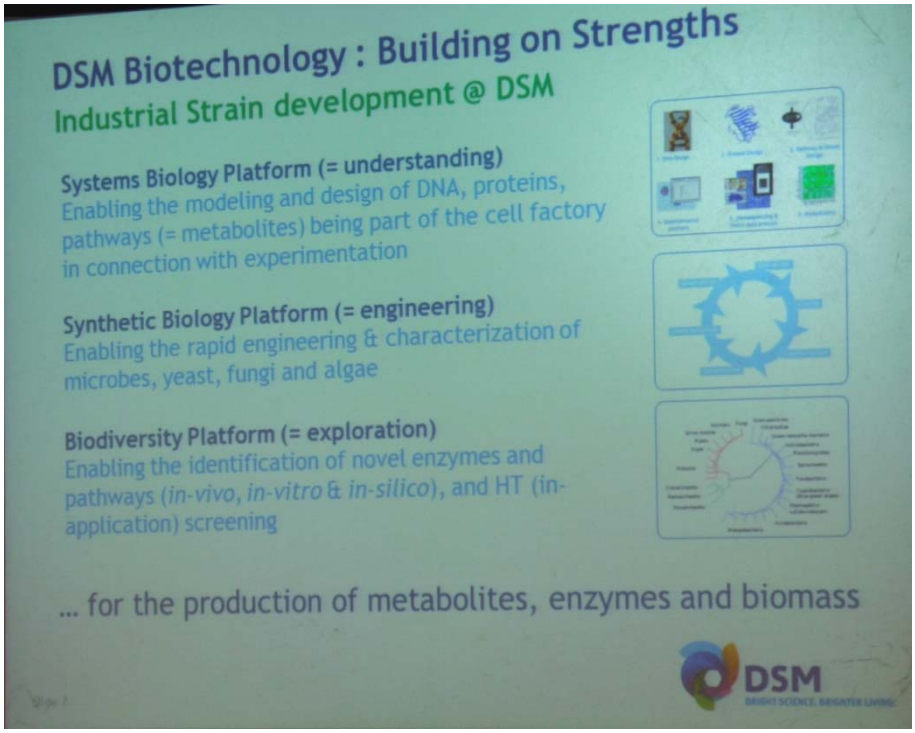
- BASF is involved in the FP7 project BioNexGen working on glucosidases, which finishes this year.

Key points discussed:

- Should the focus be on creating new products or on biotech pathways for existing ones?
- BASF's use of bioinformatics is centered around using seq. info as starting point for mutations or for new enzymes.
- BASF regards industrial enzymes as a growth field and is strengthening its position with research and acquisitions, e. g. detergent enzymes from Henkel or the proposed acquisition of Verenium.
- Dr. Baldenius made a strong point that biomass should not be inefficiently wasted for biofuels but used for generating higher-value products like surfactants and performance polymers. If energy use of biomass is desired, than electricity might make more sense than fuel.

Key points presented by Dr. van der Laan:

- DSM hosts a Biotechnology Research Center serving DSM's ambitions to provide more sustainable solution in the areas of food, pharmaceutical and white biotechnology. The center is employing about 450 scientists and technicians.
- DSM has a so-called PlugBug system technology providing various pro- and eukaryotic host strains for production of enzymes and metabolites. Examples provided were:
    - Fermentative production of a cephalosporin precursor followed by a two-step enzymatic conversion to cephalexin (instead of 13 steps);
    - Asparaginase to reduce acrylamide formation in heated food (flour, potatoes).
- DSM has developed P450 platforms based on a substrate diversity library, which is regio- and enantioselective.
- Apart from the more conventional BioIT applications, a particular challenge for BioIT in DSM perspective is both to search known sequence space as well as to generate artificial sequence space to meet the functional needs for enzymes.



Three industrial platforms presented by Dr. van der Laan, with BioIT under Synthetic Biology

Key points discussed:

- Possibilities to start with natural enzymes and then do sequence data-driven discovery and low-throughout screening based on rational processes, procedures or predictions.
- The additional effort of rational design should pay back in significant savings in respect to screening efforts and costs as well as in quality and size of the improvements to be obtained.
- Comparing this with direct strain improvement which still is much faster.

Key points presented by Dr. Ruijssenaars:

- Bioprospecting, including genomes and metagenomes, collection and analysis as useful for developing commercial products but also for new productions strains, pathways of utilization of alternative feedstocks.
- Published examples referred to were:
    - Gypsum-free lactic acid production, a main chemical used for food and non-food applications (PLA, lactides, could replace polystyrene);
    - Oxidase for 5-hydroxymethylfurfural catalysis to FDCA to replace terephthalate in polyester manufacture.

Key points discussed:

- The value of bioprospecting for uses of C5 and C6 sugars, to tolerate associated inhibitors.

- IPR and legal right related to bioprospecting leading to new isolates from soil or marine environments were shortly addressed. Using own sources ensures maximal freedom to operate.

Key points presented by Dr. Leggewie:

- evocatal is an SME working in white biotechnology which develops and produces enzymes as well as fine chemicals for the chemical and pharmaceutical industries. One core technology is the tailoring of enzymes according to industrial requirements.
- evocatal developed a signal peptide library for the production of enzymes in various hosts. Additionally it was used as a very useful toolbox for high-throughput screening, also in gram-negative hosts.
- Examples given were:
    - An enantioselective alcohol dehydrogenase for the production of R-3-hydroxy-quinuclidinol in three steps (important for pharma). Assisted by structural modelling, suitable mutations could be identified to enable industrial scale production with very high enantioselectivity and chemical purity;
    - The thermotolerance of a secreted feed enzyme could be improved from 47 to 65°C in a very short time.

Key points discussed:

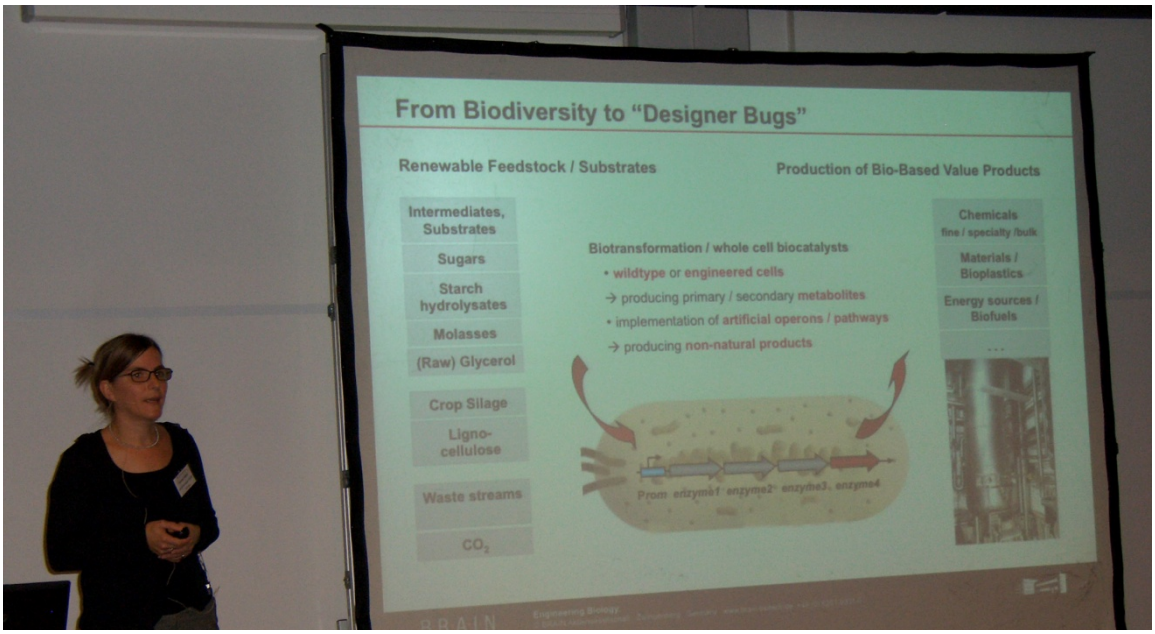
- Working with synthetic DNA (produced by another company) is becoming state-of-the-art for directed mutation/evolution work.
- For the second example provided, work is ongoing as there is still 58% of activity at 64°C and the aim is an even higher temperature tolerance.

Session 4: Databases, Discovery and Engineering

Chair: Frank Oliver Glöckner, Jacobs University Bremen	
Gabor, Esther	*From metagenomic microdiversity to designed production strains* Brain AG, Germany
Martin, Maria J.	*Database infrastructures for scientific discovery* EMBL-EBI, United Kingdom
Fernandez-Guerra, Antonio	*Exploring the dark side of the metagenomes* MPI Bremen, Germany
van den Bergh, Tom	*3DM protein super-family systems* BioProdict, The Netherlands
Vogel, Andreas	*Biodiversity, enzyme engineering and cluster screening – fast access to industrial useful enzymes* c-LEcta GmbH, Germany

Key points presented by Dr. Gabor:

- Dr. Gabor had changed her talk title to "From metagenomic (micro)diversity to designer bugs".
- The importance of an ecological and evolutionary background for developing their concepts was emphasized.
- Her company Brain AG has put a focus on combining *in silico* metagenomics with wet lab techniques, with a strong base in fundamental science and many different industrial applications. An example provided was
    - The conversion of glycerol into 1,2-propanediol (12PD) by *E. coli*, where a lacking enzyme in the metabolic pathway was identified by metagenomic screening. Based on a biosensor (for 12PD) this "missing link" was found and constitutively expressed.



Dr. Gabor presenting the overview on BRAIN's approach from substrates to products

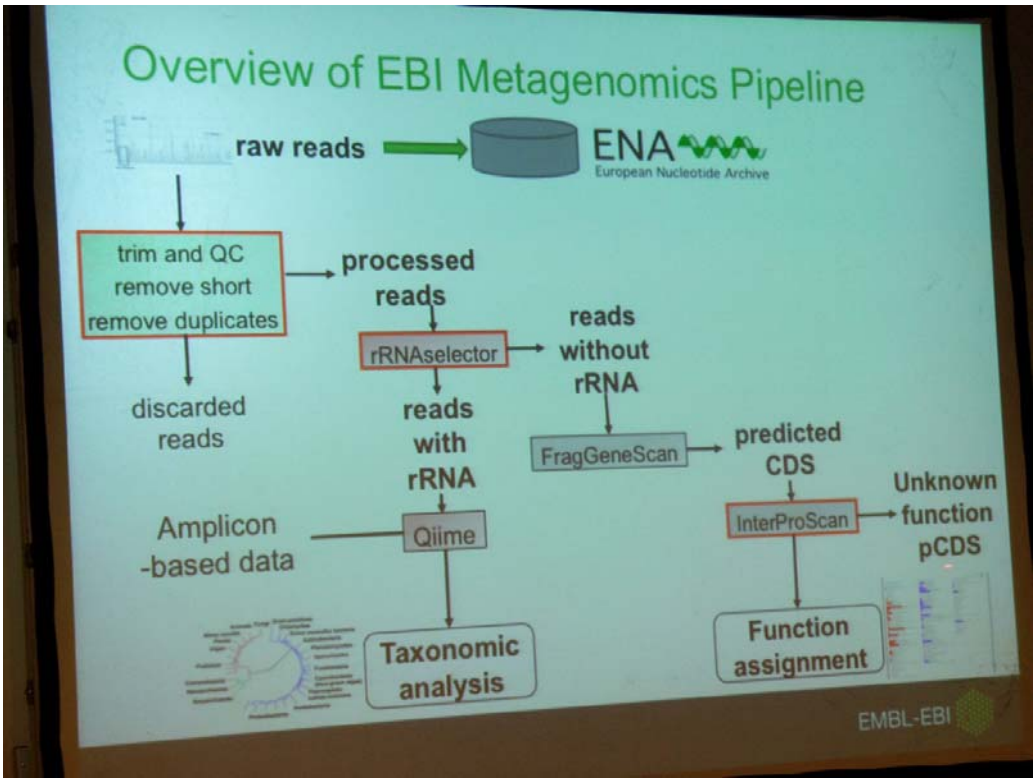
Key points discussed:

- The value of using large-insert libraries and broad host range vectors was discussed, as compared to using plasmid-based libraries with small inserts. Plasmid libraries are more readily constructed but generally require more screening effort.

Key points presented by Dr. Martin:

- A comprehensive overview of databases and other resources at the Europena Bioinformatics Institute (EBI) was provided.
- Focus was put on UniProt with 50% annotated enzymes, based on experimental data from literature; with a section on biotechnological usage.

- The EBI Metagenomic Pipeline, also called MG Portal was presented (see slide, pCDS are predicted protein coding sequences).
- EBI is promoting an Embassy Cloud infrastructure service concept, which will be secure and flexible, with the sysadmin from the user managing it.



Slide from Dr. Martin's presentation on EBI's Metagenomic Pipeline leading to phylogenetic as well as functional predictions (for full article see Hunter et al. 2013, http://zenodo.org/record/7524.)

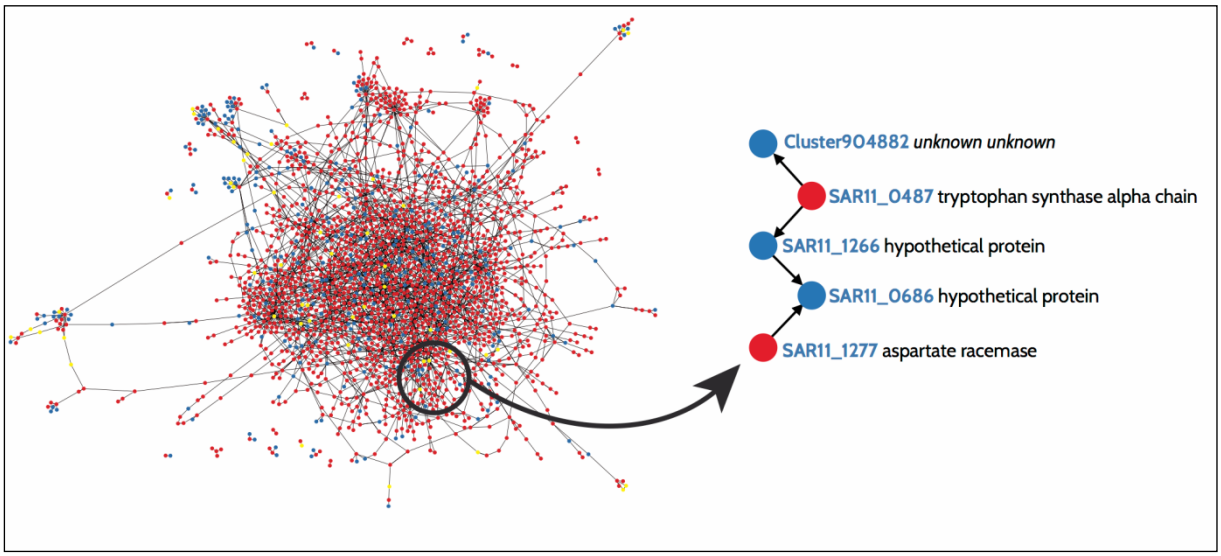
Key points discussed:

- Quality versus quantity of metagenomic short reads.
- Availability of transcriptomic data, which is a new feature, still under development, to be validated via UniProt. Here more researcher input is needed.
- A link between a Swiss structural database for small molecules and protein dbs at EBI exists, i.e. to do substrate structure searches, but incentives are needed to improve it.

Key points presented by Dr. Fernandez-Guerra:

- In a visually very appealing way it was described how to use geographic co-occurrence of genes for individual protein domains to infer information on unknown genes.
- It was presented why and how to generate network clusters and map them to KEGG.

- Their role in generating hypotheses for targeted experiments was stressed, e.g. through mapping of network clusters or putting unknown genomic data into context via BLAST.
- Of special interest to industry could be the Metagenomic Protein Domain Co-occurrence Network to infer new protein domain association and unravel the function of unknown proteins.
- A new tool called ProX (Protein eXplorer) was shown which can be used as a plugin for the Micro B3 Information System to aid researchers to explore known-unknown networks.



Slide from Dr. Fernandez-Guerra's presentation: Using the approach developed at the MPIMM any sequenced genome can be extracted from the co-occurring network of unknowns to explore the associations within the annotated genes and the hypothetical proteins, providing a valuable tool for functional hypothesis generation. In the image the Pelagibacter ubique HTCC1062 genomic network is shown with an example of how hypothetical proteins can be put into biological context.

Key points discussed:

- How 16S RNA data can be linked with protein data.
- Whether abundance is important or "filtered out".
- The importance of networks to be published for maximum usefulness to researchers, as they are computationally intensive.

Key points presented by Dr. van den Bergh:

- Bio-Prodict is a spin-off from the Univ. Wageningen specialised on three-dimensional protein visualisation and analysis (3DM).
- Several tools based on the 3DM protein superfamily database were presented, for network analysis, literature extraction and prediction to allow users to identify complex relationships and guide experimental design.

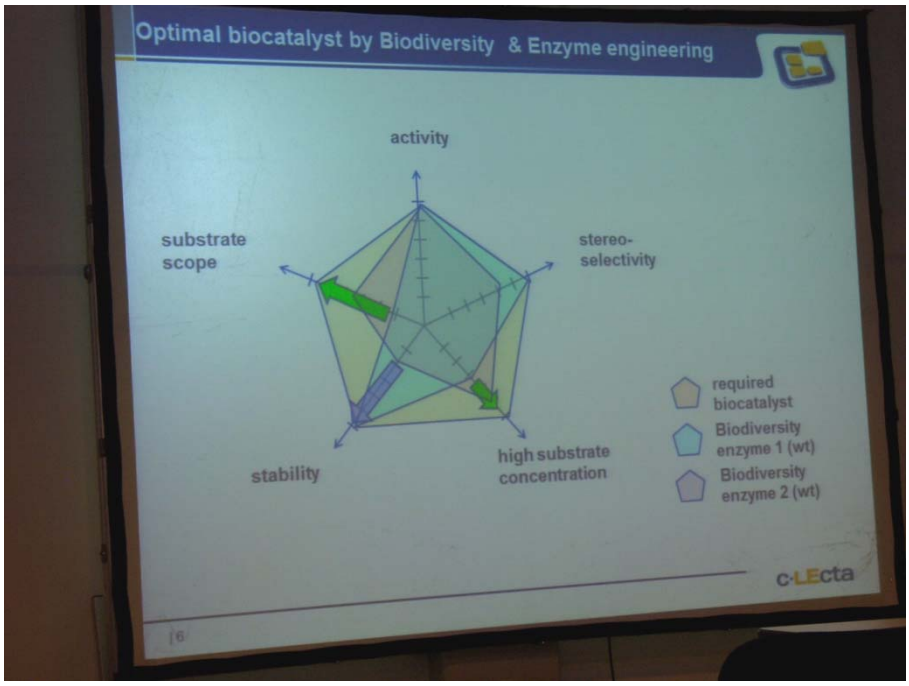
- Using P450 as example it was shown how to select hot spots for mutations based on literature data and to generate small but smart protein libraries *in silico*.

Key points discussed:

- What is allowed – not allowed based on nature, i.e. alignment-derived data.
- Whether 3DM can deal with large conformational changes. Important is that variable regions stay separated (no superfamily).

Key points presented by Dr. Vogel:

- The SME c-LEcta is specialised on rapid activity-based screening of libraries from genomic and metagenomic diversity in high throughput (10 – 100,000s of clones).
- Examples given were
    - a set of new wild type enzymes with good properties were identified for alcohol dehydrogenases using primary and secondary alcohols as substrate;
    - a heat stable asparaginase was developed for reducing acrylamide content in heat processed food, e.g. coffee beans.
- Combined with protein engineering to fulfil all required categories of properties (see slide for an example) this enzyme platform is broadly applicable.



Slide from Dr. Vogel's presentation showing five categories of properties for optimizing wild type enzymes

Key points discussed:

- The hit rate in metagenome libraries is 1:160 Mbp representing >50,000 clones. Thus 5 to 10 candidate enzymes are required resulting in about 0.5 mio. clones that are needed to be screened.
- Activity-based screens, including cluster screens are important to select for "functionally expressed" enzymes with high activity.

Panel discussion: Bioinformatics for Microbial Biotechnology and Biocatalysis

Moderator: Johanna Wesnigk (EMPA, Germany)	
Glöckner, Frank Oliver	Jacobs University Bremen, Germany
Golyshin, Peter	University of Bangor, United Kingdom (representing EU projects MagicPAH and MAMBA)
van der Laan, Jan-Metske	DSM Biotechnology Center, The Netherlands
Leggewie, Christian	evocatal GmbH, SME, Germany
Niehaus, Frank	BRAIN AG, SME, Germany (advisory board member of Magic PAH)
Wohlgemuth, Roland	Sigma-Aldrich, Switzerland

From left to right the four industry representatives on the panel: Dr. van der Laan (DSM Biotechnology Center), Dr Wohlgemuth (Sigma-Aldrich), Dr. Leggewie (evocatal GmbH), Dr. Niehaus (BRAIN AG)

Extended summary of the panel discussion along the three questions posed

1) ***Which bioinformatics tools and services are needed in the near and mid-term future in the biotechnological industry? What are the advantages when working with bioinformaticians in academia, in service companies or in house***

Views from industry:

- The role of bioinformaticians in industry is providing guidance and increasing efficiency of (experimental) approaches.
- In industry bioinformatics activities are often outsourced to specialized service SMEs (including owners of proprietary databases); relatively few bioinformaticians work within the companies represented on the panel.
- SMEs have a special need to collaborate with other companies to broaden their RTD and product spectrum by e.g. linking genetic/genomic information with substrates and other parameters (pH, temperature, stability, etc.).
- Bioinformatics is still an academic discipline close to basic research and not yet directly profitable for industrial needs. At this stage it fits best within RTD projects and not as an integral part of the production processes.
- Nevertheless, academic knowledge is needed to solve customer problems and to create innovative products.

Note: it became clear after the discussions that a common definition of the term bioinformatics was missing and should have preceded the discussion, i.e. bioinformatics dealing with the design of databases and computational algorithms to analyse biological data with the primary goal to increase the understanding of biological processes.

The point was raised by academia: what kind of training in bioinformatics is useful for a company, e.g. a master degree in computational biology to train people that can work with companies?

Views from industry:

- Training workshops are especially important for SMEs. As they cannot afford to hire specialist(s) for bioinformatics, they need to train their own staff in the use of bioinformatics tools. This is needed to collaborate with external partners and universities, and to extend their horizon to new techniques.
- SMEs like BRAIN AG and evocatal GmbH are open to joint Bachelors, Masters and PhD theses done on their premises and co-supervised by them.
- Large companies need specialists with some practical background. They need to know how to apply and use bioinformatics in the lab, not to develop methods. This is left to service SME as they are often more specialized in bioinformatics.
- Networking with other companies is important.

2) ***Involvement in research projects: what are bottlenecks in exploring, prospecting and exploitation of (marine) biodiversity (infrastructure, proof-of-principle, samples, upscaling, etc.)?***

Views from academia:

- There is big demand for high quality data at all levels. These are needed for databases especially to close the gap between input of sequences and functional characterization of proteins, also for industrial purposes. Besides bioinformatics predictions, experimental validation is needed. Model organisms can help in this respect.
- Submission of curated functional information to EMBL-EBI is needed. Bioinformatics can only proceed if all data are in public databases. Public databases can only be as good as the data the users are willing to submit and share.
- Sharing of data was a major issue in EU project negotiation with industry partners.
- Data need to be digital available to be machine-readable for easy integration into databases.

Views from industry:

- Most data from large companies are published in e.g. patent literature.
- SMEs like BRAIN AG are creating knowledge, thus their purpose is also to share their data.
- Every company has to create economic value whereas academia needs to produce publications.
- Wouldn't global economy incrementally benefit if we are able to agree with the scientific community to create a new global functional database for enzymes? Industries and scientific community should develop standardization of this database together.

Existing special databases (input from Dr. Glieder, ACIB):

https://muteindb.genome.tugraz.at (specific mutein database on kinetic data of catalyzed reactions, containing thousands of reactions of different enzyme classes.)
www.brenda-enzymes.org (enzyme database including enzyme functional data.)

3) ***What are lessons learnt during industry-academia collaboration research projects in biotechnology? (Final statements)***

Dr. Niehaus (BRAIN AG): we are closely linked with academia, as we started as an academic spin-off. Our company has a history in German- and EU-funded research projects. Furthermore the company is involved in two large BMBF Innovation Alliances (ZeroCarb FP and NatLifE 2020, the latter in capacity as coordinator. Thus we are heavily dependent on cooperation with companies and academia.

Dr. Leggewie (evocatal GmbH): we have been involved in some big projects like MAMBA, with a few of them finished now. We achieved very promising results in almost all projects which highlights how important international cooperations are, particularly for SMEs. We would like to make available parts of the results, share and continue working on them. Focussing our attention on few, selected topics in RTD projects is important, in order to balance short- and long-term interests. For example, we coordinate one German funded research project on new applications for enzymes in the fields of textiles, concrete and detergents.

Dr. Wohlgemuth (Sigma-Aldrich): our company cooperates globally and is involved in a lot of research projects. The collaboration within European is increasing. One of the main questions is where in the world should we create new jobs? How can European research funding help here. We suggest to focus on exciting areas with a potential for break through: get a good group together and set joint industry-academia goals to address open innovation potential in the best possible way.

Dr. van der Laan (DSM): DSM also endorses open innovation. The idea is to increase collaborations with other companies as well as with academia and centers of expertise: if the expertise is outside, you should go outside and collaborate. We are involved in several programmes/projects involving academia and other companies for the development of new products and new technologies. Joint work on technologies instead of products is less IPR sensitive so better for cooperation although opportunities for the generation of new IP will remain to be an important aspect. Participating in EU projects was seen as very demanding considering the organizational and legal aspects, which made entering these projects less attractive. The EU took measures to improve, which may make it again more attractive to join. Care needs to be taken to agree on a clear focus and not to diverge otherwise the company may loose interest.

Dr. Golyshin (Univ. Bangor): we have made different experiences with different companies involved in our projects (MAMBA, MagicPAH, MICROB3 and ULIXES). Some companies are not allowed to share results (due to their national terms of IPR). In the just finished MAMBA project only 200 of ~2000 clones are characterized, so many more could be made available. More output from public funds seems possible but how, if IPR has to be safe guarded. A lesson learnt from the involvement in research projects is to avoid redundancy among companies.

Dr. Glöckner (Jacobs University Bremen): speaking as co-founder of a bioinformatics SME called Ribocon GmbH: the involvement in a research project is important for companies. This creates the possibility to explore new fields and to increase their networking capacity. In regard to EU projects “the first one is the worst”, thereafter chances for networking and opportunities for joint research override administrative efforts.

Annexes

Annex 1: Handouts for first Micro B3 Stakeholder Workshop, February 2013, Brussels

Agenda

Participants

Annex 2: Programme book for first Micro B3 Industry Expert Workshop, October 2013, Groningen

Introduction to Micro B3 and MetaExplore

Agenda

Abstracts of Talks and Posters

Participants

Annex 1.
Handouts for first Micro B3 Stakeholder Workshop, February 2013, Brussels

AGENDA:

WEDNESDAY 27TH FEBRUARY

10:00 – 10:15 Welcoming Remarks
Frank Oliver Glöckner, Max Planck Institute for Marine Microbiology Jacobs University Bremen (Coordinator of MICROB3)

10:15 – 10:30 Introduction of the background of the workshop and panelists
Tom Dedeurwaerdere, Université catholique de Louvain (WP8 leader)

THE LEGAL BACKGROUND FOR MICROB3 MODEL ABS AGREEMENT

10:30 – 10:50 The legal framework for ABS in comparison of the Convention on Biological Diversity (and the Nagoya Protocol) and United Nations Convention on the Law of the Sea (UNCLOS)
Gerd Winter, University of Bremen

10:50 – 11:10 COFFEE BREAK

11:10 – 11:30 Provider state ABS legislation applicable to access to marine microorganisms: the example of Kenya
Evanson Chege Kamau, University of Bremen

11:30 – 11:50 User state legislation: the example of the EU proposal for an ABS Regulation
Thomas Greiber, IUCN

11:50 – 12:10 An original Instrument, the CIESM Charter on Marine Genetic Resources Access and Benefits Sharing
Michele Barbier, CIESM

12:10 – 12:30 Questions

12:30 – 13:30 LUNCH BREAK

THE SCIENTIFIC AND BIOINFORMATICS BACKGROUND FOR MICROB3 MODEL ABS AGREEMENT

13:30 – 13:50 How do taxonomic and genomics data bases work and adapt to ABS?
Chris Lyal, Department of Entomology, Natural History Museum, London

13:50 – 14:10 How do material collections of micro-organisms work and adapt to ABS requirements?
Tom Dedeurwaerdere, Université catholique de Louvain

14:10 – 14:30 How can provider state researchers be involved in research and development on marine microorganisms?
Chris Bowler, scientific coordinator of Tara Oceans expedition

14:30 – 14:50 Which research and development results might be candidates for what kind of intellectual property protection?
Jakob K. Kristjansson, Prokazyme, Reykjavik, Iceland

14:30 – 15:00 Questions

15:00 – 15:30 COFFEE BREAK

EXPERIENCES WITH ACCESS TO GENETIC RESOURCES FOR COMMERCIAL RESEARCH AND DEVELOPMENT (Panel discussion)
Moderator: Johanna Wesnigk, Environmental and Marine Project Management Agency, Bremen

15:30 – 16:30 Representatives from EU consortia and from industries
BlueGenics
PharmaSea
SeaBioTech
Aquapharm
NanotedMARIN GmbH
Prokazyme

INTRODUCTION OF THE ANALYSIS AND DISCUSSION OF MICROB3 MODEL ABS AGREEMENT

16:30 – 17:00 MICROB3 ABS model agreement: access, utilization of genetic resources and benefit-sharing
Caroline v. Kries, University of Bremen

17:00 – 17:30 MICROB3 ABS model agreement: transfer to third parties and dissemination policy
Arianna Broggiato, Université catholique de Louvain

17:30 – 18:30 Questions and voluntary division of the participants into the two panels

19:30 Dinner

THURSDAY 28th February

PRACTICAL CASE STUDIES

9:00 – 9:20 The designing and practical implementation of permits for accessing geothermal microbes in Iceland
Jakob K. Kristjansson, Prokazyme, Reykjavik, Iceland

9:20 – 9:40 Exploring the options for ABS of Marine Genetic Resources in the Antarctic Treaty System
Roser Puig Marcó, University of Barcelona

ANALYSIS AND DISCUSSION OF MICROB3 MODEL ABS AGREEMENT WITHIN TWO PARALLEL PANELS

9:30 – 13:00 **PANEL A. Access to and utilization of genetic resources**
PANEL B. Transfer of genetic resources and data management

13:00 – 14:00 Lunch break

14:00 – 15:00 Summary of the outcomes of the panels by the facilitators

15:00 – 16:00 Concluding remarks

Participants' List First Micro B3 Stakeholder Workshop

Name	Affiliation
Altuğ Atalay, Mustafa	Ministry of Food, Agriculture and Livestock, Fisheries and Aquaculture Department, Turkey
Arrieta López de Uralde, Jesús María	IMEDEA -Instituto Mediterraneo de Estudios Avanzados, Mallorca, Spain
Aydin, Ilhan	Ministry of Food, Agriculture and Livestock, Central Fisheries Research Institute, Turkey
Barbier, Michele	CIESM - Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée, Monaco
Bicak, Mesude	Oxford e-Research Centre, University of Oxford, UK
Bowler, Chris	Centre National de la Recherche Scientifique, Ecole Normale Supérieure, Paris -Tara Expeditions
Broggiato, Arianna	BIOGOV Unit, Université catholique de Louvain, Belgium
Chiarolla, Claudio	IDDRI - Institut du développement durable et des relations internationales, Paris
Day, John	University of Strathclyde, Glasgow, UK - SeaBioTech Project
Dedeurwaerdere, Tom	BIOGOV Unit, Université catholique de Louvain, Belgium
Edrada-Ebel, RuAngelie	Sciences University of Strathclyde, Glasgow, UK - SeaBioTech Project
Elbour, Monia	INSTM - Institut National des Sciences et Technologies de la Mer, Tunisia
Field, Dawn	Oxford e-Research Centre, University of Oxford, UK
Glöckner, Frank Oliver	Max Planck Institute for Marine Microbiology, Jakobs University, Bremen, Germany
Greiber, Thomas	IUCN Environmental Law Centre, Bonn, Germany
Husrevoglu, Sinan	The Scientific and Technological Research Council of Turkey, TUBITAK, Turkey
Kamau, Evanson Chege	University of Bremen, Germany
Kristjansson, Jakob K.	Prokazyme EHF, Reykjavik, Iceland - BlueGenics Project
Lyal, Chris	Natural History Museum, London, UK
Marteinsson, Viggo	Matis ltd,Reykjavik, Iceland
Mazuranok, Laurence	University of Strathclyde, Glasgow - SeaBioTech Project
McMeel, Oonagh	eCOAST Marine Research, Belgium - PharmaSea Project
Mearns-Spragg, Andrew	Aquapharm Biodiscovery Limited, UK - PharmaSea Project
Müller, Werner	Institute of Physiological Chemistry of the Mainz University Medical Center, Germany - BlueGenics Project
Nardello, Ilaria	Ireland's Marine Biotechnology Programme, Marine Institute Oranmore, Co. Galway, Ireland
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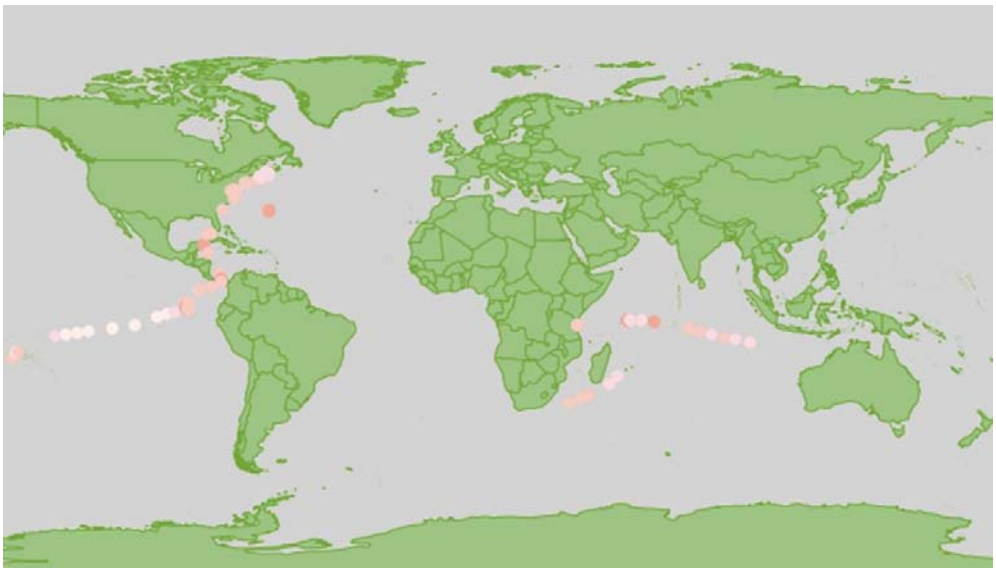
Puig, Roser	Departament de Dret i Economia Internacionals, Facultat de Dret, Universitat de Barcelona
Rosales, Kattia	National Biodiversity Institute INBIO, Costa Rica
Scaria, Arul	BIOGOV Unit, Université catholique de Louvain, Belgium
Schroeder, Heinz-Christoph	NANOTECMARIN GMBH, Mainz, Germany - BlueGenics Project
Siam, Rania	Biology Department, American University of Cairo, Egypt
Tianbao, Qin	Wuhan University School of Law, China - University of Göttingen
Verkleij, Gerard	Fungal Biodiversity Centre - UTRECHT, The Netherlands - MIRRI project
Veske, Erdinç	Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Politics, Turkey
von Kries, Caroline	University of Bremen, Germany
Wesnigk, Johanna	EMPA - Environmental & Marine Project Management Agency, Bremen, Germany
Winter, Gerd	University of Bremen, Germany

First Industrial Expert Workshop
MicroB3-MetaExplore

Harvesting Environmental Genomes
for the Development of Biocatalysts

October 14 – 15, 2013

De Oosterpoort
Trompsingel 27 / Groningen
The Netherlands



Harvesting Environmental Genomes for the Development of Biocatalysts

October 14-15, 2013, De Oosterpoort, Groningen, The Netherlands

Dear Participants,

On behalf of the organizers I am pleased to welcome you to this MicroB3-MetaExplore Industrial Expert Workshop. The workshop is a dissemination activity of the two EU FP7 projects, which target the development and use of tools for exploring microbial (meta) genomes.

We hope that the joint participation of industrial and academic scientists will stimulate the intensive exchange of different ideas and views, and thereby contribute to the identification of innovative approaches in microbial genomics and bioinformatics for use in biotechnological applications.

I look forward to a fruitful workshop and wish you a pleasant stay in Groningen.

Dick Janssen
(Chair Organizing Committee, University of Groningen)

First Industrial Expert Workshop MicroB3-MetaExplore

Harvesting environmental genomes for the development of biocatalysts

October 14 - 15, 2013

Cultural Center De Oosterpoort, Groningen, The Netherlands
Trompsingel 27, 9724 DA Groningen

The enormous microbial biodiversity of marine environments and soils offers an almost unlimited resource of new enzymes and bioactive compounds. The possibilities to explore this diversity for the development of new applications in industry and medicine are rapidly increasing due to the use of innovative tools in (meta)genomics, bioinformatics and high-throughput analysis.

This Industrial Expert Workshop brings together academic and industrial scientists with the aim to exchange current approaches for the discovery and engineering of microbial activities for use in biocatalysis, synthetic biology and biomass processing. Opportunities and challenges of novel tools for understanding complex genome data and for the discovery of enzymes, pathways and bioactive compounds by high-throughput methods will be discussed.

Speakers

Kai Baldenius, BASF, Germany
Tom van den Bergh, BioProdict, NL
Antonio Fernandez-Guerra, MPI Bremen
Fergal O'Gara, BIOMERIT, Cork, Ireland
Esther Gabor, Brain AG, Germany
Anton Glieder, Univ. of Graz, Austria
Dick Janssen, Univ. of Groningen, NL
Kristiina Kruus, VTT, Finland
Maria Martin, EBI, UK
Christian Leggewie, Evocatal, Germany
Jan-Metske van der Laan, DSM, NL
Marnix Medema, MPI Bremen, Germany
Michail Yakimov, IAMC, Italy
Harald Ruijssenaars, Purac/Corbion, NL
Andreas Vogel, c-LEcta GmbH, Leipzig, Germany
Liz Wellington, Univ. of Warwick, UK
Roland Wohlgemuth, Sigma-Aldrich, Switzerland

Chairs

Jan-Dirk van Elsas, Univ. of Groningen, NL
Frank Oliver Glöckner, Jacobs University Bremen, Germany
Peter Golyshin, University of Bangor, UK
Dick Janssen, Univ. of Groningen, NL
Johanna Wesnigk, EMPA Bremen, Germany

Organisation

Dick Janssen, Univ. of Groningen (chair)
Jan-Dirk van Elsas, Univ. of Groningen (MetaExplore)
Frank-Oliver Glöckner, MPI Bremen (MicroB3)
Johanna Wesnigk, EMPA (MicroB3)
Sandra Haan and Tamara Hummel, Univ. of Groningen

MicroB3

The EU FP7 project MicroB3 (Biodiversity, Bioinformatics, Biotechnology) aims to develop innovative bioinformatic approaches in order to make large-scale data on marine microbial genomes and metagenomes accessible for ecosystems biology and biotechnological exploration. MicroB3 builds upon a highly interdisciplinary consortium of 32 academic and industrial partners, comprising renowned experts in bioinformatics, computer science, biology, ecology, oceanography, bioprospecting and biotechnology, as well as legal aspects.

MicroB3 benefits from earlier and ongoing European sampling campaigns that explore long-term marine research sites. A strong link between oceanographic and molecular microbial research is being established, which integrates global marine data with knowledge on microbial biodiversity and functions. MicroB3 will provide innovative open-source software for data-processing, -integration, and -visualisation, which will support the discovery of new useful functions.

Within MicroB3, Work Package 7 targets the development of biotechnological applications based on the growing amount of marine genomic data and novel bioinformatic tools. New software tools and experimental approaches are applied to assign gene function to unknown genes, to harvest enzymes from marine metagenomes and to discover bioactive compounds encoded by biosynthetic gene clusters in marine organisms. The resulting candidate genes are used to perform targeted confirmatory experiments.

To broaden the range of enzymes and bioactive compounds that can be discovered, experimental approaches involving gene library and micro-culture techniques, as well as dedicated screening methods are also used. The enzyme discovery work, which is the main topic of the current workshop, will focus on reactions involved in the synthesis of secondary metabolites that may be applied as bioactive products and on enzymes that transform synthetic compounds of biotechnological importance.

The MicroB3 project is supported by the OCEAN.2011-2 program, grant no. 287589.

Coordinator
Prof. Frank-Oliver Glöckner, Max Planck Institute for Marine Microbiology, Bremen

Lead WP 7
Prof. Dick Janssen, University of Groningen

MetaExplore

Metagenomics for Bioexploration – Tools and Application

The MetaExplore project is driven by both industrial demand and state-of-the-art environmental metagenomics technologies. It develops and applies advanced molecular tools that allow the cloning and sequencing of the metagenomes of microbial communities of selected soil and aquatic habitats, followed by educated activity- and sequence-based screenings for, and analyses and engineering of, target enzymatic activities. These desired activities are derived from industrial demand, and focus in particular on improved enzymes involved in the biodegradation of recalcitrant and xenobiotic molecules, including novel chitinases, ligninases, and aerobic and anaerobic dehalogenases.

A strong focus is placed on the mobilome, that is, the collective pool of mobile genetic elements carried by a microbial community, as there is strong evidence that the frequency of occurrence of genes encoding the desired enzymatic activities is raised in this gene pool. Selected genes/operons found to encode useful novel enzymatic functions are analyzed at the molecular level and expressed in optimized expression vectors in suitable hosts.

Subsequently, the key enzymes are characterized as to structure and function (kinetics), and used in directed evolution or protein engineering experiments to enhance or extend their activities to a practical level.

MetaExplore is supported by the EU FP7 program KBBE-2007-3-3-05 under grant agreement no. 22625.

Coordinator
Prof. Jan-Dirk van Elsas, Unit Marine Microbiology, University of Groningen

First Industrial Expert Workshop MicroB3-MetaExplore

General information

Venue
Cultural Center De Oosterpoort, Groningen, The Netherlands

Trains Amsterdam Schiphol → Groningen and vice versa
(http://www.ns.nl/en/travellers/home)

There are two intercity trains per hour that provide a direct link from Amsterdam-Schiphol to Groningen and vice versa. The travel time is about 2½ h.
Trains leave from Schiphol to Groningen at 03 (direct line) or 33 (change in Zwolle) past the hour. From Groningen to Schiphol at 16 min (change in Zwolle) or 46 min (direct line) past the hour.

Registration and welcome
The program will begin on Monday, October 14 with registration from 09.00 to 10.00. The first session will start at 10.00. Tuesday we will start at 08.45 with session 4 and closure is at 14.45.

Badges
Participants are kindly requested to wear their badges all times throughout the workshop.

Internet
Wireless internet is available: instruction will be provided on site.

Dinner
Monday evening dinner is scheduled at 19.00 at the Restaurant in De Oosterpoort.

Harvesting Environmental Genomes for the Development of Biocatalysts

Industrial Expert Workshop, a joint dissemination activity by the MetaExplore and MicroB3 EU Projects

October 14-15, 2013, De Oosterpoort, Groningen, The Netherlands

PROGRAM

Monday, 14 October 2013

9:00-10:00	**Registration**
10:00-12:30	**Session 1: New enzyme discovery and engineering**

Chairs: Jan-Dirk van Elsas, University of Groningen & Frank-Oliver Glöckner, MPI Bremen

10.00-10.15	Chairs opening remarks, introduction MicroB3 and MetaExplore FP7 projects
10.15-10.55	Anton Glieder, Austrian Centre of Industrial Biotechnology (ACIB), Graz: Novel enzymes from transcriptomes
10.55-11.35	Kristiina Kruus, VTT, Finland: Discovery of novel cellulases and hemicellulases from unique metagenomic libraries
11.35-12.10	Liz Wellington, University of Warwick, UK: The studying enzymes in soil using metaproteomics
12.10-12.40	Michail Yakimov, IAMC, Italy: Organisms and enzymes from deep-sea hyper-saline lakes
12:40-13:40	**Lunch**
13:40-16:00	**Session 2: Bioinformatics and discovery**

Chair: Peter Golyshin, University of Bangor

13.40-14.20	Fergal O'Gara, BIOMERIT, Cork, Ireland: Current trends in marine biotechnology
14.20-15.00	Roland Wohlgemuth, Sigma-Aldrich: New biocatalysts for industrial applications
15.00-15.30	Marnix Medema, MPI Bremen: Genomics-based discovery of bioactive compounds
15.30-16.00	Dick Janssen, Univ. Groningen: Computational approaches in enzyme discovery and engineering
16:00-16:30	**Break**

16:30-18:00 **Session 3: Industrial needs**

Chair: Dick Janssen, University of Groningen

16.30-17.10 Kai Baldenius, BASF, Germany: Industrial biocatalysis - enzyme catalysis for efficient chemical production

17.10-17.50 Jan-Metske van der Laan, DSM: Enzyme discovery for biocatalysis and synthetic biology

17.50-18.25 Harald Ruijssenaars, Purac/Corbion, The Netherlands: Bioprospecting – an industrial perspective

18.25-19.00 **Drinks (De Oosterpoort)**

19:00 **Dinner (De Oosterpoort)**

Tuesday, 15 October 2013

08:45-12:30 **Session 4: Databases, discovery and engineering**

Chair: Frank Oliver Glöckner, Jacobs University Bremen

08.45-09.20 Christian Leggewie, Evocatal, Germany: Improvement of biocatalysts for industrial applications

09.20-09.55 Esther Gabor, Brain AG, Germany: Experimental strategies for metagenomic exploration

09.55-10.30 Maria J. Martin, EMBL-EBI: Database infrastructures for scientific discovery

10.30-11.00 **Break**

11.00-11.30 Antonio Fernandez-Guerra, MPI Bremen, Germany: Exploring the dark side of the metagenomes

11.30-12.00 Tom van den Bergh, BioProdict: 3DM protein super-family systems

12.00-12.30 Andreas Vogel, c-LEcta GmbH, Leipzig: Biodiversity, enzyme engineering and cluster screening – fast access to industrial useful enzymes

12:30-13:30 **Lunch**

13:30-15:00 **Panel Discussion: Bioinformatics for Microbial Biotechnology and Biocatalysis**

Chair: Johanna Wesnigk, EMPA Bremen

A panel of industrial participants and EU project partners will discuss the use of bioinformatics and genomics tools for discovering new enzymes and pathways for industrial biotransformations and other biotechnological applications. Confirmed participants: Peter Golyshin (Univ. Bangor), Christian Leggewie (Evocatal), Frank Niehaus (BRAIN AG), Jan-Metske van der Laan (DSM), Frank-Oliver Glöckner (MPI Bremen).

15:00 **Closure**

Abstracts – Oral Presentations

Novel enzymes from transcriptomes

Aleksandra Mitrovic[1], Laura Naeaetsaari[2], Aleksandra Fuchs[2], Anton Glieder[2]

[1] Austrian Centre of Industrial Biotechnology (ACIB), and [2] DK Molecular Enzymology @ Institute of Molecular Biotechnology, TU Graz, Petersgasse 12, A-8010 Graz, Austria

In addition to bacteria plants, animals and fungi provide a vast and often complementary diversity of enzymes. Transcriptome sequencing, analysis and expression in yeasts as well as direct functional screening of normalized cDNA expression libraries now provide a similar simple and quick access to new enzymes from eukaryotes as from the bacterial world. New bioinformatics tools to search for conserved protein domains and a new small expression plasmid for library construction and gene expression in fission yeast have been generated.

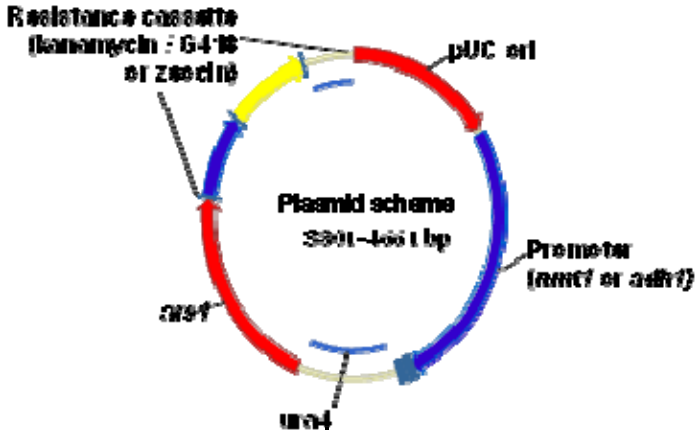


Fig. 1. New fission yeast expression plasmids for cDNA library expression.

Discovery of Novel Cellulases and Hemicellulases from Unique Metagenomic Libraries

Paul Bromann[1], Simo Ellilä[2], Kaisa Marjamaa[2], Mari Nyyssönen[2], Merja Itävaara[2], Anu Koivula[2] and Kristiina Kruus[2]

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Microorganisms encompass the largest resource of metabolic and genetic diversity encountered on Earth. Since a major part of the microbiota in natural ecosystems (often 95 - 99%) is not culturable, metagenomic approaches are vital for discovery of target enzyme activities or DNA sequences. A need for a more efficient enzymes for different applications is evident. Especially the increasing use of lignocellulosic for fules and chemicals necessitates discovery and development of novel lignocellulolytic enzymes. We report the use of metagenomic techniques to screen for novel cellulase and hemicellulase activities. Metagenomic DNA was isolated from unique environmental samples and controlled composts enriched with various lignocelulosics. Fosmid and plasmid-based expression libraries were constructed and screened for cellulase activity based on dyed carboxymethyl cellulose (CMC) and Azurin-crosslinked xylan. This approach was successful and various novel enzymes were discovered in the screen.

The Studying enzymes in soil using metaproteomics

Elizabeth M.H.Wellington, Ashley Johnson-Rollings, Helena Wright and Victoria Hibberd

School of Life Sciences, University of Warwick, Gibbet Hill, Coventry, CV4 7AL, UK

Methods for direct molecular analysis of the soil microbiota and in particular those microorganisms in the rhizoplane and rhizosphere have advanced considerably since the revelations achieved by PCR amplification of 16S rRNA gene targets in soil defining the uncultured majority of bacteria. Whole genomes will soon be revealed following metagenomic analysis of agricultural soil but extensive functional analysis eludes soil microbiologists and has only been successful in the study of well-defined groups such as nitrifying bacteria[1] and methylotrophs[2]. Metatranscriptomics is demanding *in vitro* but in soil is particularly difficult as bacterial mRNA can have a limited stability of less than 6 seconds[3]. Protein is an ideal target for soil analysis due to the known stability of extracellular enzymes and other proteins in soil, which may relate to interactions with organic matter[4]. Over the last 30 years extraction protocols were developed to attempt extraction of protein initially to assay enzymes and have included freezing, boiling, bead-beating, sonication and dialysis combined method but with low protein recoveries. No robust method for analysis of the extracellular proteins has emerged. Data on metaproteomics in soil provided lists of proteins detected and identified from the directly extracted proteome of all soil bacteria[5, 6] but in one case the soil was heavily inoculated with *Pseudomonas putida* or *Arthrobacter chlorophenolicus.* Wang et al.[6] recovered proteins from the rhizosphere and of the 189 identified proteins 107 originated from plants, 72 from bacteria or fungi (43 from bacteria) and 10 from fauna. Bacterial proteins were involved in protein metabolism, secondary metabolism, nucleotide metabolism, signal transduction and resistance and the majority came from *Proteobacteria.* These methods extract proteins from all cells and the majority of proteins will be intracellular and the extracellular proteins will not be distinguished. It will be impossible to determine the exoproteome from such studies. Our aim is extract the total metaproteome but also to include the extracellular proteins in the soil sample, as enzymes involved in breakdown of polymers must be in the extracellular milieu in order to act on these substrates[7]. In addition the periplasm of gram negative bacteria will be extracted in the metaexoproteome and this provides data on uptake mechanisms expressed in soil. Wilmes and Bond[8] pioneered protein extraction from environmental samples and argue that proteomics can detect physiological responses to changes in environmental conditions. We are confident that the soil metaexoproteome (SMEP) and total metaproteome (MP) will provide valuable information on the range and source of enzymes involved in the breakdown of natural polymers in soil, plant litter[9] and also on proteins involved in plant-microbe interactions. Metagenomic approaches to expression screening for enzymes involved in chitin and lignocellulose degradation provide a useful source of sequence data to assist in identification of proteins and recovery of genes.

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[9] Voriskova J, Baldrian P. Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME J. 2013; **7**(3): 477-86.

Organisms and Enzymes from the Deep-sea Hypersaline Anoxic Lakes

Michail M. Yakimov[1], Violetta La Cono[1], Manuel Ferrer[2] and Peter N. Golyshin[3]

[1]Institute for Coastal Marine Environment, CNR, Spianata S.Raineri 86, 98122 Messina, Italy, [2] Institute of Catalysis, CSIC, Marie Curie 2, 28049 Madrid, Spain, 3 School of Biological Sciences, Bangor University, ECW Bldg Deiniol Rd, Bangor, Gwynedd LL57 2UW, UK

Eight deep-sea hypersaline anoxic lakes (DHALs) have recently been discovered on the seafloor of the Mediterranean Sea. Nascency of these peculiar hydrological formations is related with dissolution of the ancient salt deposites, called Messinian evaporites. The surface of these lakes lies 3.0-3.5 km below sea level and the salinity of the brines is five to thirteen times higher than that of seawater. As follows, the sharp density gradient between their hypersaline brines and the upper seawater acts as a stable barrier for the exchange of oxygen.

All of these factors make the DHALs one of the most polyextreme environments on Earth. Some major discoveries have been made in the last years on very peculiar microbial life adapted to such hostile environments. According to the recent findings, DHALs contain much more diverse prokaryotic assemblages with a surprisingly high number of novel prokaryotic candidate divisions than the shallower anoxic marine hypersaline basins. This enigmatic microbiota represents yet-hidden biodiversity belonging to at least 12 novel bacterial and archaeal candidate divisions named as MSBL, Mediterranean Sea Brine Lakes. Remarkably, members of enigmatic bacterial candidate division KB1 dominate the DHAL brines. These organisms are among deeply branching bacteria considered to be the nearest to the node dividing the prokaryotic kingdoms. Until now OMICs studies in DHALs are rare and only very recent investigations have identified a number of key microbial proteins in DHALs and linked them to essential metabolic processes and environmental adaptations [1].

To explore and exploit the genomic diversity hidden in DHALs microbiota we performed generating and screening of metagenome expression libraries. New enzymatic diversity, exemplified by five esterases, was found in microbes present in DHABs, specifically in the brine:seawater interface of the Urania West Basin [2]. Two of which function under the extreme conditions of DHAB brines, and a one possesses an adaptive structure-function configuration that confers high catalytic activities under a very wide range of physicochemical conditions. These DHAB esterases are promising candidates for the synthesis of optically pure biological active substances that provide access to pharmaceutical intermediates.

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Current Trends in Marine Biotechnology

Fergal O'Gara

BIOMERIT Research Centre, Department of Microbiology, University College Cork, Ireland

Marine ecosystems constitute a diverse plethora of environmental niches ranging from the extreme hypothermal to the arctic cold and from the high pressure deep sea bed to the almost atmospheric ocean surface. For many years the presence and role of microorganisms in marine ecosystems has been underappreciated. However new technologies are beginning to uncover the vast diversity of marine organisms that exist in the marine environment. With this diversity comes an unparalleled range of bioactivities which are offering solutions to key biotechnological needs. These include applications in society such as generating new antimicrobials, biocatalysts, and functional foods to name a few. Parallel developments in genomic and bioinformatic technologies are increasing our capacity to decode the bioactivity of the marine resource. Innovative high throughput functional screens coupled with diverse metagenomic libraries are providing important alternatives to enhance the current state of the art.

The BIOMERIT Research Centre (BRC) Marine Biotechnology Programmes employ advanced technologies to decipher the bioactive potential of marine organisms, primarily from the Porifera (marine sponge) clade of Eukarya. Culturable and unculturable approaches have yielded important and novel bioactivities. These include; a novel lantibiotic, subtilomycin, identified in marine Bacilli, with activity towards multidrug resistant pathogens; a barotolerant cold-active lipase adapted to high salt and solvents; a screen to detect novel C-P bond cleaving activity for biotransformations; as well as additional enzymatic and anti-infective compounds.

Maintaining the success of these marine programmes relies on continuous technological and innovative advances, both in addressing current challenges and in exploring new possibilities. The BRC is involved in a number of EU programmes that are addressing current and future challenges in marine biotechnology. These includes issues such as the culturability of marine organisms as well as the development of effective heterologous expression systems. In tandem with this, exciting new approaches combining synergies between biocatalysis and the diversification of marine small molecules are also being explored for the development of innovative molecular therapeutics directed towards cancer and infection treatments in clinical medicine.

New Biocatalysts for Industrial Applications

Roland Wohlgemuth

Research Specialties, Sigma-Aldrich, Buchs, Switzerland

Biocatalyst toolboxes have been attractive in many classical industrial applications, where single step biocatalytic reactions have replaced existing chemical reactions because of overall benefits [1-4]. Biocatalysts are key for achieving catalytic asymmetric synthesis applications in sustainable chemistry due to their extraordinary selectivity and inherent chirality [5-7]. The interface with organic chemistry is thereby important [8-10] and a comprehensive biological and chemical view and analysis of the overall synthetic system is useful [11]. The toolboxes need to be filled with biocatalysts enabling the application of classical biochemical reactions in organic synthesis, catalytic versions of known reaction types as well as new types of chemistry in order to remain competitive with the rapid developments in sustainable chemistry. In the area of analytical applications the focus is on the instrumental interface with the detection methodology, single step biocatalytic reactions, biocatalysts with high selectivity, reliability, stability and complexity reduction.

One area where integrated approaches of the molecular sciences of synthesis and analysis with the engineering sciences are particularly important is the synthesis of metabolites [12]. Metabolites are prerequisites for the functional assays of biocatalysts and to discover novel biological functions. The expansion of enantiomerically pure metabolites enables better stereochemical characterizations of pathway steps in healthy biological systems as well as in inborn errors of metabolism and acquired diseases. Natural metabolic pathways offer numerous inspirations for the design of synthetic reaction sequences and for the application of biocatalytic tools towards the synthesis of densely and differentially functionalized small molecular weight natural products. Biocatalytic tools interfaced with organic chemistry have been useful for the catalytic asymmetric synthesis of a number of metabolites along central biochemical pathways, which up to now have been difficult to access. In addition to the design of the synthetic sequence and selective reactions, stability issues, selective product recovery and purification methodologies are key for preparing the final products.

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Genomics-based Discovery of Bioactive Compounds

M.H. Medema[1], K. Blin[2], A. Fernandez-Guerra[1], R. Kottmann[1], F.O. Glöckner[1,7], P. Cimermancic[3], T. Paalvast[4], D.D. Nguyen[5], P.C. Dorrestein[5], M. Fischbach[3], T. Weber[2], R. Breitling[6] & E. Takano[6]

[1] Microbial Genomics and Bioinformatics Research Group, Max Planck Institute for Marine Microbiology, Bremen, Germany,2 Mikrobiologie/Biotechnologie, Eberhard Karls Universität Tübingen, Tübingen, Germany, 3 Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco, USA, 4 Department of Microbial Physiology, University of Groningen, 5 Departments of Chemistry/Biochemistry and Pharmacology, UCSD, San Diego, USA, 6 Faculty of Life Sciences, Manchester Institute of Biotechnology, University of Manchester, Manchester, United Kingdom, 7School of Engineering and Science, Jacobs University Bremen, Germany

Microbes from the environment are a rich source of bioactive compounds with potential pharmaceutical applications. In recent years, computational methods have become more and more important to identify these compounds. Within the scope of the EU project Micro B3 (Biodiversity, Bioinformatics, Biotechnology, www.microb3.eu), the effective use and implementation of these methods can be of great use to link identified compounds to biosynthetic gene clusters and to help design effective sampling strategies.

With antiSMASH (http://antismash.secondarymetabolites.org), we developed a key computational pipeline that has the capability of identifying biosynthetic genomic loci covering the whole range of known secondary metabolite compound classes [1,2]. Given a genome or metagenome, antiSMASH automatically identifies gene clusters within the nucleotide sequences. Moreover, it performs chemical structure predictions, domain analysis of modular enzymes, phylogenetic analysis and comparative analysis with homologous gene clusters [3]. Novel extensions allow the direct matching of identified gene clusters with mass spectrometry data [4,5]. We are currently devising new strategies to employ these and other tools to guide metagenomic sampling and gene cluster reconstruction from metagenome libraries.

Combining antiSMASH with a generic algorithm that identifies genomic regions involved in small molecule biosynthesis, we have recently performed a global quantitative and comparative analysis of biosynthetic gene clusters in all microbes [6]. The results offer new leads towards the identification of novel classes of molecules and thus show that a computational approach can radically transform the search for novel chemical scaffolds that could lead to the development of novel antibiotics and antitumor drugs.

To ensure effective experimental characterization of the identified gene clusters at large scales, we have designed high-throughput synthetic biology methodologies for activity screening and industry-scale production [7-9].

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Computational Approaches in Enzyme Discovery and Engineering

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Many biocatalytic processes rely on microbial enzymes that catalyze catabolic reactions in their natural host. The discovery and the tailoring of microbial enzymes for industrial conversions often involve time consuming approaches such as library construction and high-throughput screening. We are exploring the integration of computational approaches in enzyme discovery and protein engineering to improve enzymes for synthetic processes, targeting properties such as thermostability and solvent tolerance, expanded substrate range, and regio- or stereoselectivity.

For improving thermostability, we developed a strategy (FRESCO, framework for enzyme stabilisation by computation) in which point mutants and disulfide bond mutants are first generated in silico and evaluated by calculating differences in free energy of folding. Only variants that passed computational tests were expressed and tested experimentally. Confirmed beneficial mutations were combined, again after testing the multi-site mutants by molecular dynamics simulations. This allowed spectacular thermostabilisation of an epoxide hydrolase, a dehalogenase, an electron-transfer protein, and a peptide amidase, in only two or three rounds of evolution.

We also explore the use of homology models for predicting the catalytic activity of putative enzymes discovered by metagenome sequencing. Whereas modeling is not sufficiently accurate to identify with high certainty the activity of individual enzymes, the use of docking and molecular dynamics simulations may offer a means for ordering and ranking promising sequence variants for experimental verification.

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Industrial Biocatalysis - Enzyme Catalysis for Efficient Chemical Production

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In the last decades the application of enzymes for enantioselective syntheses has made its way to industrial use. BASF has led this development with its Chipros® product line of chiral intermediates. However, the horizons for enzymatic catalysis are now going far beyond pharma intermediates. Examples for non-chiral volume chemical application will be given.

Enzyme Discovery for Biocatalysis and Synthetic Biology

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The demand for biologically processed food, for more natural ingredients and supplements, pharmaceuticals, antibiotics and sustainable production methods for chemicals and fuels is growing rapidly. To enable this growth the competence to find and develop the proper enzymatic functionalities is a key success factor. Looking at the major traditional applications of industrial enzymes like for example detergents and starch processing the number of different enzymes that is used is rather limited when considering the large biodiversity that is out there. To keep up with new trends and developments, new enzyme backbones were screened from nature, but in most cases the old backbones were successfully adapted by protein engineering to fulfill the extended performance requirements. It just might reflect the ability of protein engineering to improve a single property within a small sequence space without affecting other proven beneficial properties. Natural variants screened from biodiversity usually cover a much wider sequence space. As a consequence they usually differ in respect to multiple properties which are critical for industrial enzymes e.g. expression, solubility, production yield, stability. On the other hand the larger sequence space may rather be an advantage when looking for new enzyme functionalities to enable new innovative concepts for the application of enzymes.

The need for new enzymes is not limited to isolated enzymes. Synthetic biology aims at designing and optimizing new revolutionary *in vivo* synthetic pathways to produce new and existing compounds in a more sustainable way. Many of these pathways need new enzyme functionality to perform. Although the modern tools to access biodiversity have improved considerably and open up an almost unlimited number of activities and specificities, in many cases these natural templates need further optimization, likely because evolutionary pressure was absent towards the specificity, selectivity, and efficacy needed in industrial processes. To illustrate this some examples will be presented which are related to the development of new enzyme applications and processes at DSM.

Bioprospecting – an Industrial Perspective

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Corbion is a worldwide leader in biobased food ingredients, chemicals and polymer building blocks. Examples are lactic acid and lactic acid derivatives (lactate salts, lactic acid esters, lactides), glycolides and caprolactone monomers.

In a continuous effort to increase customer value, product and process development at Corbion are directed towards reducing cost and improving functionality while enhancing environmental credentials. One of the research tools to achieve such goals is bioprospecting, i.e., sampling the natural environment for biological resources that have a commercial value. For a fermentation-based company like Corbion, these resources may entail new or improved production strains, pathways or enzyme activities that enable the production of new, industrially relevant molecules or the utilization of alternative feedstocks.

A recent example from literature is the isolation of a strain of *Cupriavidus basilensis* from an environmental sample via enrichment on 5-hydroxymethylfurfural (HMF). Upon analysis of the HMF catabolic pathway, a unique oxidase was discovered that catalyses the oxidation of HMF to 2,5-furandicarboxylic acid (FDCA) [1]. FDCA is a potential green replacement of terephthalate in the manufacture of polyesters that are applied in, *e.g.*, softdrink bottles and textiles. The compound was identified as one of the top-12 most promising biomass-derived chemicals [2, 3].

In the presentation, the relevance and utility of bioprospecting approaches in a broad sense will be discussed and put into perspective from an industrial angle.

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Improvement of Biocatalysts for Industrial Applications

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The application of biotechnology for the production of biofuels, polymers and fine chemicals has gained more and more importance during the last two decades. This trend was supported by the consumer´s acceptance for „biological“ products and the increasing efforts of the chemical industry to establish resource-saving and sustainable production processes. In the focus of the developments are biocatalysts which are often the product itself. When improved to fit into process conditions enzymes can outmatch chemical catalysts in terms of speed, costs, purity and sustainability. Nevertheless biotechnological processes compete directly with classical processes developed and optimized over decades and run in written off plants. But those processes that have been established so far show that efficient application of biocatalysis is possible. And the number of processes is increasing constantly. There are two main reasons for this. The first reason is a tremendous increase in efficiency concerning the microbiological tools that stand behind the biocatalyst that is used in a conversion on industrial scale. Not too long ago scientists were happy when a wild-type enzyme could be overexpressed in an *E. coli* strain and showed activity not only against its natural substrate. Today it is state of the art to tailor an enzyme according to the requirements of the used substrate and the anticipated reaction conditions as well as to produce this catalyst in an almost unlimited scale. The second reason is the foreseeable scarcity of raw materials and the needs for alternative processes and synthetic approaches resulting from that.

Experimental Strategies for Metagenomic Exploration

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Industrial "white" biotechnology is regarded as a key technology in modern industrialized societies for ensuring a sustainable economic future. It combines efficiency, the use of renewable resources and environmental friendliness to produce high-value products. In this respect, the introduction of highly efficient enzymes into heavily engineered microorganisms - so-called "designer bugs" - can improve existing processes or enable novel product ideas, thus paving the way to a knowledge-based bioeconomy.

The replacement of traditional chemical processes by multi-step biosynthetic reactions in sustainable processes is based on fairly recent technological innovations in the fields of microbial genomics, metagenomics, *in vitro* and *in vivo* evolution. An ever increasing number of industrially relevant enzymes have been, for instance, discovered by the use of wet lab and *in silico* metagenomic techniques. The non-paralleled diversity of microbial enzymes even seems to be vaster than expected as recently suggested by the observation of molecular microdiversity of single enzymes in natural habitats. This finding emphasizes the continued need of fundamental research as well as technology development in the field of metagenomic enzyme discovery.

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Database Infrastructures for Scientific Discovery

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Biomolecular databases collect and store biological information and provide essential platforms for research infrastructures for life scientists. The EMBL-EBI maintains the world's most comprehensive range of freely available and up-to-date molecular databases, from nucleotide sequences to full systems. Our services let scientists share data, perform complex queries and analyse the results in different ways. Amongst these, the UniProt database [1] describes the genes and proteins products from many organisms and describes their functions, role in biological pathways, chemistry reactions and their involvement in diseases and the biotechnology industry. The InterPro resource [2] provides functional analysis of proteins by classifying into families and predicting domains and important sites. The metagenomics resource stores and analyses metagenomics data providing a stable infrastructure for these data for public re-use. These databases provide a framework for improved understanding of microbial data and for scientific discovery. I will describe the information contained in these databases, the methods used to annotate them and some of the ways they may be exploited to support scientific discovery and innovation.

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Exploring the dark side of the metagenomes

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Metagenomic environmental surveys, like the Global Ocean Survey (GOS), generated a huge amount of genetic data and allow performing more holistic approaches to study marine ecosystems. Moreover, metagenomics proofed being valuable in discovering missing links in marine biological processes. Besides expanding our limited view on the diversity of the known protein universe, metagenomics also revealed a large number of genes of *unknown* functions. These can be further classified into I) *known unknowns* like the domains of unknown function (DUF) and II) *unknown unknowns*, putative coding sequences without any hint of potential function. We will present a novel approach to extract valuable information from the co-occurrence of individual protein domains involved in biological processes using Graphical Models. Using an integrative approach, we combine the knowledge of the known protein domain families and 16S ribosomal DNA with the *unknown unknowns* to explore the GOS metagenome. As a result, we were able to reveal new associations in biological processes within known protein families and between known protein families and *unknowns*.

3DM protein super-family systems

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3DM protein superfamily databases are systems that contain vast amounts of different data types available for a complete protein superfamily, such as sequences, structures, mutation data (collected from different sources, such as mutation databases and literature), structure derived data (e.g. protein-protein interactions, ligand-protein interactions, h-bridges, salt-bridges, solvent accessibility, etc), and alignment derived data (conservation, correlated mutations, etc). 3DM contains and is connected to many different tools for easy analysis of the data, such as CorNet (a correlated mutation network analysis tool), Mutator (a literature mutation extraction tool), Validator (a mutation prediction tool), Utopia (an intelligent PDF reader that integrates scientific literature with superfamily data in the 3DM database), Yasara (a state-of-the-art protein structure visualization tool that integrates structure data with data from the 3DM database). A consistent numbering scheme is applied to all sequences and structures, which is based on a highly accurate structure bases multiple sequence alignment. All tools use this numbering scheme enabling easy data transfer and comparison. This enables the user to find complex relations between different data types making this coherent system highly suitable to guide experiment design from different scientific research fields.

3DM has successfully been utilized in protein engineering (smart library design; improved thermostability and enantioselectivity, change enzyme specificity, increase enzyme activity), drug design (reveal the different binding modes of inhibiting- and activating small molecules, inhibitor design), DNA diagnsotics (highly accurate mutagenesis predictions) all published in peer review articles.

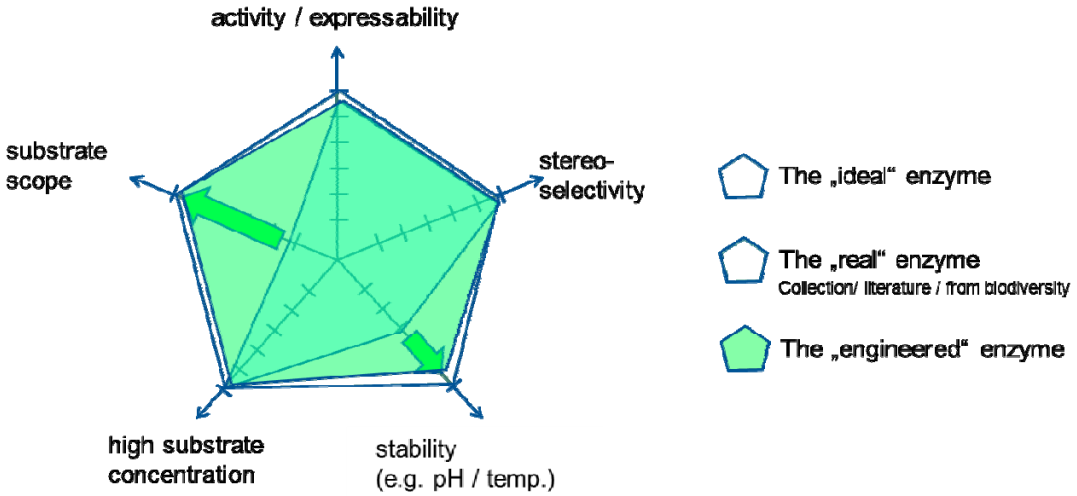
Biodiversity, Enzyme Engineering and Cluster Screening – Fast Access to Industrial Useful Enzymes

Andreas Vogel and Rico Czaja

c-LEcta GmbH, Leipzig, Germany

Customized enzymes with improved performance tailored for specific requirements are still in high demand. The analysis of natural biodiversity provides a huge potential for the identification of new enzymes needed for the development of new biotechnological products or for the optimization of current industrial processes. Combining of the enormous potential of natural diversity with the ability to design highly representative genomic and metagenomic libraries together with a rapid and innovative screening method can open access to a huge amount of novel and specific biocatalysts. c-LEcta´s proprietary Cluster Screening enables a rapid screening of libraries from genomic and metagenomic biodiversity on an activity basis. New enzymes are identified by activity in a throughput of a few 10,000 to several 100,000 clones per screening run for virtually all enzyme classes.

We could demonstrate that ADH-screenings with primary and secondary alcohols as substrates have led to a set of different and new enzymes depending on the used substrate when using the same library for screening. The novel wt enzymes obtained from biodiversity had perfect properties regarding activity and selectivity. They were further optimized by enzyme engineering to broaden their substrate scope. The combination of biodiversity and engineering thus yielded a unique and broadly applicable enzyme platform.



Abstracts – Posters

Identification of promiscuous hydrolases from metagenomic libraries screened in microfluidic droplets

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Microfluidic droplet technology offers an alternative to classical screening systems with reaction volumes reduced to the picoliter scale [1,2]. Besides decreasing the cost of screening campaigns by reducing consumables use needed, it increases the throughput by up to three orders of magnitude [3] to well above a million assays per hour.

The development of a miniaturized single cell lysate assay in water-in-oil droplets [4] has been the starting point of the development of a new screening platform for functional metagenomics. Small insert metagenomic libraries from soil and cow rumen were transformed into *E.coli* and single cells encapsulated into droplets (volume: 3 pL) together with lysis agents and fluorogenic substrates. Upon cell lysis, metagenomic plasmids are trapped in the same compartment together with the released catalysts constituting the essential genotype-phenotype linkage for a screening system. After incubation of the droplets "off-chip" for up to 3 days, the emulsion is re-injected into a sorting chip where fluorescent droplets are separated from the negative non-fluorescent ones, allowing the direct selection of plasmids coding for active catalysts.

The very high-throughput of the microfluidic technology allowed us to screen a library of more than 10^6 variants in 2 hours, while still overscreening the library by more than 10 times. The characterization of one specific hit (selected when screening for sulfatase activity) confirmed the isolation of a new promiscuous [5] sulfatase exhibiting activity toward sulfate monoester ($k_{cat}/K_M = 1.14\text{x}10^5\ M^{-1}s^{-1}$) and phosphonate monoester ($k_{cat}/K_M = 2.75\text{x}10^1\ M^{-1}s^{-1}$). When screening for phosphotriesetrases, we isolated 8 new proteins from different superfamilies displaying phosphotriesterase activity, an activity thought to have recently evolved from a promiscuous activity [6]. These hits would not have been predicted by database searches based on their sequences, demonstrating the power of our functional microdroplet assay.

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The Protein Factory: from biocatalysis to biomedicine "Metaexplore cases"

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"The Protein Factory" Research Center [1] has been established by the Politecnico of Milano (Dept. CMIC G. Natta), the University of Insubria in Varese (Dept. Biotechnology and Life Sciences) and the CNR in Milan (Institute of Chemistry of Molecular Recognition) to promote basic and applied research activities on proteins of biotechnological relevance (red, white, green biotech).

The aim of "The Protein Factory" consists in offering an integrated laboratory for the production and characterization of native and engineered recombinant proteins useful in the different fields of biotechnology. Presently, the proteins produced/engineered by the different units belonging to the Center are:

- Acylases (glutaryl acylase, cephalosporin acylase, lipoglycopeptide and lipodepsipeptide acylases)
- Oxidases (D-amino acid oxidase, glycine oxidase, proline oxidase, cholesterol oxidase, histamine oxidase, diamine oxidase, laccases)
- Proteases (bacterial proteases, HIV-1 protease, D,D-carboxypeptidases)
- Cellulases, chitinases, peroxidases
- Aminotransferases
- Phospholipase D
- *Mycobacterium tuberculosis* antigens (Ag85B, TB10.4), human pLG72, Hsp70, α-synuclein.

In the frame of the European MetaExplore project, the laboratory of Microbial Biotechnology as a unit of "The Protein Factory", has worked on the cloning, expression and characterization of two different proteins:

- a D,D-carboxypeptidase (named VanY) involved in a novel glycopeptide resistance mechanism in *Nonomuraea* sp. ATCC 39727 [2].In this case, *Streptomyces* spp. have been used as unconventional hosts for protein expression developing a novel platform to produce heterologous proteins sourced in high G-C DNA content bacteria [3];

- and the chitinase Chi18H8 whose encoding gene was isolated from a suppressive soil metagenomic library by the group of Prof. Sara Sjöling of the Södertörn University, School of Natural Sciences and Environmental Studies, Sweden [4, 5]. Interestingly this enzyme has antifungal properties and it will be possibly developed as a biocontrol agent for phytopathogens.

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Cultivation and Detection of Halometabolite Producing Bacteria from the Marine Sponge *Halichondria panicea*

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Sponges are ancient sessile invertebrates that have been around since 700 million years ago. Sponges are a prolific source for the discovery of bioactive compounds ranging from anti-infective, antibacterial, anticancer and antifungal. Often the true producers of such compounds were found to be sponge-associated microorganisms. In the marine environment, sponges often produce halogenated secondary metabolites, particularly alkaloids. Halogenases have been shown to play a significant role in the biosynthesis and introducing bioactivity to many secondary metabolites. The majority of halogenated metabolites in the marine environment contain bromine, whereas chlorinated compounds are most commonly synthesized by terrestrial organisms. Previously, occurrence of brominated compounds has been recorded in the marine sponge *Halichondria panicea*. In our recent study, a diverse collection of bacteria comprising several genera has been isolated from *H. panicea.* Our aim in this project is to screen this culture collection for the presence of flavin-dependent halogenase genes, test their anticancer activity and elucidate the structure of the halometabolite(s). Ninety nine different isolates from *H. panicea* were subjected to the halogenase screening. An initial PCR screening using degenerate flavin-dependent halogenase primers confirms the presence of a halogenase in at least 4 different bacterial phyla isolated from the sponge. This study underlines the genetic potential of sponge-associated bacteria from *H. panicea* for the production of halometabolites.

Computational Redesign of Epoxide Hydrolase for Enantioselective Synthesis

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The ability to redesign naturally occurring enzymes to a desired regio- and enantioselectivity would have great benefits for the synthesis of specialty chemicals. Current methods mostly rely on directed evolution. Here we present a method in which most experimental screening is replaced by computational methods. An essential element is that the reactivity of a bound substrates is estimated by calculating the frequency of occurrence of near-attack conformations during molecular dynamics simulations of enzyme-substrate complexes [1]. This method was applied to the redesign of a thermostable variant [2] of limonene epoxide hydrolase for conversion of cyclopentene oxide to optically active diols.

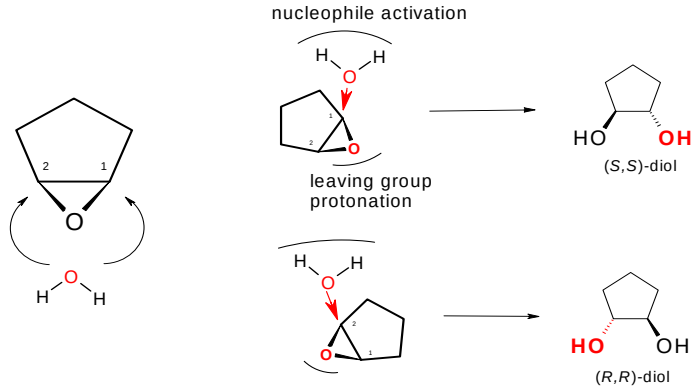

Fig. 1. Source of product enantioselectivty in epoxide hydrolase. The positioning of the substrate relative to the nucleophilic water will determine regioselectivity, which in turm determines if (*R*,*R*) or (*S*,*S*) product is formed.

Twenty-nine epoxide hydrolase variants were computationally designed and tested experimentally. Of the variants that were designed to be (*S*,*S*)-selective, 63% was correctly predicted, and of the variants designed to be (*R*,*R*)-selective 85% was correct. Both for (*S*,*S*)-diol and (*R*,*R*)-diol enantioselectivities exceeding E=80 were observed. The final enzyme variants obtained were as enantioselective as those obtained by a comparable directed evolution experiment, but while experimentally screening far less variants.

Acknowledgement: this work is supported by the EU FP7 projects Metaexplore (grant agreement no. 22625) and Kyrobio (grant agreement 289646).

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Amylomics: Metagenomics for Retrieval of Carbohydrate Active Enzymes

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The goal of the AMYLOMICS EU-project is to develop new robust enzymes for the starch and carbohydrate industries. For this purpose an efficient platform technology for enzyme screening, called *Targeted Metagenomics*, was developed. The process comprises microbial enrichment techniques, massive parallel 454 FLX sequencing and the Roche-NimbleGene's sequence capture technology. In the project, anaerobic and microaerophilic enrichments of environmental samples from geothermal habitats in Iceland were prepared, using starch derivatives and other carbohydrate substrates. Thus, metagenomes of a moderate complexity, high diversity and enhanced evenness were generated, suitable for FLX sequencing and enriched in starch or other carbohydrate utilizing organisms. The biological diversity of the metagenome samples was monitored using 16S rRNA sequence analyses. Few metagenomes of appropriate diversity and evenness were selected for the high throughput sequencing. Due to the extent of the metagenome data, assembly of the sequence reads yielded high number of contigs and residual singletons with partial open reading frames (orfs). To improve the data, probes close to the ends of the sequence contigs and singletons were designed and constructed and used to capture flanking sequences from the original metagenome fragment library. Reassembly of the metagenome sequence data with the sequence capture reads resulted in merged and new contigs and increased number of complete orfs, including carbohydrate genes. Several starch modifying enzyme genes identified in the Amylomics metagenome database were cloned and expressed in *E. coli*. Subsequently, the recombinant enzymes were characterized and their products analyzed. In the poster, the *Targeted Metagenomics* procedure and the outcome will be described and as well as properties of few novel amylolitic enzymes.

Acknowledgement: This work was supported by the EU FP7 grant 265992: AMYLOMICS.

Prospecting extreme costal environments for carbohydrate enzymes and polysaccharides applying genomic and metagenomic sequencing

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In the SeaBioTech project various extreme biotopes have been prospected for polysaccharide processing enzymes including marine intertidal or submerged areas. These areas are covered by a profusion of algal species containing great variety of complex recalcitrant polysaccharides as well as rich invertebrate fauna with "unconventional" structural polysaccharides. Organisms in these environments need to compensate for the deleterious effects of extreme conditions, e.g. wave action, temperatures, in osmolarity, low pH or temperature, high radiation and fluctuations from one extreme to another. Many of the adaptive strategies adopted by the organisms depend on polysaccharides in one way or another -in special properties of structural polysaccharides, in special polysaccharides that mediate adhesion to inert surfaces or polysaccharides that counteract wave action; and, in macroalgae, invertebrates and microorganism in production of highly hydrated extracellular polysaccharides which help to deter desiccation. Marine contain all the lignocellulose sugars to a varying degree depending on the species, but in addition "rare sugars", in appreciable quantities e.g. deoxy sugars, sugar acids and sugar alcohols. They are complex, often branched and highly substituted. These polysaccharides are abundant source of carbon, and marine microbes from these unique biotopes are therefore expected to be valuable source of scientifically interesting and industrially applicable polysaccharide processing enzymes.

The unique, intertidal Yngingarlindir (YL) ponds on the South West coast of Iceland are fed by an affluent from a geothermal power plant and seawater. This is an example of a relatively stable marine geothermal habitat that differs to other such sites in Iceland and elsewhere in that its salinity is approximately that of the sea. Geothermal coastal intertidal hot springs are usually fed by fresh hot water from terrestrial sources and with much lower salinity.

The uniqueness of the site if reflected in the large variety of novel species and genera detected and isolated. Of 56 marine extremophile isolates, from the site, 49 are preserved in Matis culture collection. These were isolated at diverse culture conditions in connection with O_2, temperature and salinity. The isolates were identified to the species or genus level revealing that the majority of the isolates belong to 17 novel genera and/or species within several phyla within the Eubacteria. 45% of the strains belonged to α-Proteobacteria, 19% to the CFX group, 19% to γ-Proteobacteria and the rest to the Chloroflexi and Firmicutes phyla. MATIS has now and will present data on the rich and variable content of polysaccharide processing enzymes in these organisms. A special database/processing and comparison software was developed by MATIS for storing data and allowing easy access and genome searches. The annotation comprised classification of genes into categories, subcategories and subsystems. Genes detected potentially encode enzymes capable of degrading complex marine polysaccharides, including chondroitin sulfate, chitin, laminarin, fucoidan and alginate.

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Pan genome of the *Thermus* species

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Thermal habitats are unique in many aspects and many of their features make them attractive and perhaps ideal model systems for studies of microbial divergence and speciation. Microbial speciation may be more easily observable in geothermal habitats than in other systems since they are geographically confined and comparatively rare on a global scale. Therefore, they can be considered as islands in the ecological sense, separated by large distances and physicochemical dispersal barriers. It has been postulated that geographical isolation is likely to be a major factor in causing and enhancing the divergence of microbes. It promotes divergence of populations due to founder effects and different geothermal regions may have different selective pressures and unique species compositions and interactions. Geographic isolation creates opportunities for isolated evolutionary events, local lateral gene transfer occurrences and local environmental adaptations by periodic selection [1, 2]. In this project we are using powerful methodologies of genomics and cultivation independent molecular analysis to detect and investigate fundamental species differences that may explain the ecological adaptations of *Thermus* species, as well as their niches and roles in geothermal ecosystems. 18 genomes sequenced by Matís from different species along with 10 publically available genomes will be used to define the core genome of the genus *Thermus*, resolve species genomic differences and define species specific peripheral genes that might distinguish between and explain different ecological adaptations of these species. An in-house software for pangenomic analysis has been developed and accessory data on distribution of *Thermus* in 32 hot springs obtained by FLX 16S rRNA sequencing from water, biomats and sediment samples has been obtained.

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Targeted illumina sequencing of 16S rRNA and functional marker genes to decipher microbial communities involved in biogeochemical cycling processes

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The biogeochemical fluxes of the six major elements of the biosphere, i.e., H, C, N, O, S, and P, are mainly controlled by distinct microbial communities that intimately interact to create organized flows of energy, electron donors and acceptors. Because the microbial communities do not exist at random and present a high level of organization and networking to control ecosystem functioning, the development of holistic approaches is required for a systems-level understanding of community structure and function as well as the interactions between community members and their influence on the overall behavior of the system. Thanks to the emergence of next-generation sequencing (NGS) technologies and the associated bioinformatics pipelines, we are now able to grasp complex microbial processes at the community level with unprecedented high-depth resolution. Compared to other NGS approaches (e.g., 454 pyrosequencing), the illumina sequencing platform HiSeq2000™ is one of the most efficient technology in terms of read quality and quantity with a sequencing output/run up to 600 Gb and a maximum number of reads/run of 6×10^9. Targeted amplicon sequencing focusing on the bacterial/archaeal 16S ribosomal RNA (phylogenetic marker) is commonly used to decipher community structure. For instance, custom-designed primers targeting the 16S rRNA hypervariable region V4 are used to generate amplicons of about 415 bp (illumina-based 16S-tags (*iTags*)) that can simultaneously cover both bacterial and archaeal communities. 150-nucleotide paired-end multiplex sequencing is then performed with the HiSeq2000™ platform followed by *in silico* assembly of paired-end reads and identification of operational taxonomic units (OTUs). Another emerging approach relies on the targeted sequencing of functional marker genes encoding key enzymes involved in biogeochemical cycling processes (e.g., genes encoding nitrogenases (*nif* gene; nitrogen fixation), hydrogenases ([NiFe] hydrogenase gene; H_2 oxidation), carbon monoxide (CO) dehydrogenases (*coxL* gene; CO oxidation) or reductive dehalogenases (*rdhA* gene; dechlorination). Although different bioinformatics pipelines and toolkits need to be developed, the combined sequencing of phylogenetic and functional marker genes will shed light on specific active functional guilds (including low-abundance community members) and is poised to provide systems-level models for functionality prediction and enable optimized management of microbial communities involved in the major biogeochemical cycles.

Discovery and Characterization of Aminotransferases for the Biosynthesis of Non-Proteinogenic Amino Acids

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Non-proteionogenic amino acids, such as β-amino acids, are very important molecules that posses a broad range of applications, from drug synthesis to polymer design. Although biocatalytic routes have been explored for the production of such amino acids, contemporary eco-friendly routes are far from optimal because they are usually based on kinetic resolutions, which suffer from 50% yield limits. Thus, enzymatic asymmetric synthesis of such eantiopure amino acids using non-chiral starting materials will be an attractive alternative. Suitable enzymes are aminotransferases, ammonia lyases, and aminomutases. However, a broad biodiversity is lacking within these types of enzymes, which are also limited in terms of substrate tolerance.

Our lab investigates the discovery of new enzymes, using screening of (meta)genomic sequences for selection of primary target genes, high-throughput homology model generation of the encoded enzymes, as well as high-throughput molecular docking simulations of *in silico* substrate libraries. The results should yield methods for identifying catalytic functions of putative aminotransferases, and for selecting from genomic sequences those that encode enzymes that are strong candidates for a biocatalytic conversion. The methods are tested with small systems, such as known aminotransferases, in oder to discover critical steps in the computational and bioinformatics procedures.

In the current study, we present the aforementioned protocol applied to the discovery and characterization of transaminases with putatively broad substrate tolerances. Overall, the aim is to provide a foundation for the computational prediction of substrate scope of environmentally- and industrially-relevant enzymes from marine origins.

Metagenomic screening for aromatic contaminant biodegradation enzymes using viral systems that facilitate genes expression.

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Functional metagenomics is an important tool for the discovery of new activities of interest. One major limitation of functional metagenomics is gene expression of the metagenomic library in the bacterial host.

We have developed two expression systems on the pCC1FOS fosmid that allow heterologous genes expression of metagenomic DNA when using specialised *Escherichia coli* strains derived from EPI300-T1^{R} (Epicentre). To drive metagenomic gene expression, one system uses T7 phage RNA-polymerase, a fast and highly processive enzyme able to transcribe through many bacterial transcriptional terminators, whilst the other uses the lambda phage N antitermination system in an attempt to prevent transcription termination at terminators that RNA-polymerase may find within the metagenomic DNA. We found that the use of the viral expression systems increased the number of the positive hits by 6-fold when selecting metagenomic genes providing carbenicillin resistance (100 mg L^{-1})[1].

Once the efficiency of the expression systems was proven, we focused our work in the construction of metagenomic libraries from a polluted soil in order to screen for different activities of interest involved in biodegradation of aromatic contaminants. The resulting metagenomic library consisted of 185,000 clones.

By spraying catechol or 1,2-dihydroxybiphenyl on the grown colonies on LB agar plates, we identified 59 different clones coding for ring-cleavage extradiol dioxygenases (EDOs)(yellow colonies). These clones were tested for activity towards 5 different substrates and found 2 with very high relative activity towards the recalcitrant molecule 4-chlorocatechol. 22 extradiol dioxygenase coding genes have been identified among the clones and ascribed to the two types of enzymes. Phylogenetic analysis of the type I EDOs metagenomic sequences identified highly divergent sequences representative of two new subfamilies.

By screening transformation of tryptophan into indigo (blue pigment) we found 34 different clones with ring-hydroxylating oxygenase activity. 16 clones were sequenced and found that all bear genes coding for different types of monooxygenases, including one bearing a complete dibenzothiophene biodegradation operon. The clones are being characterised for activities generating molecules of interest in different industrial sectors. So far, we have found one clone able to transform naphthalene into 1-naphthol, a precursor of different insecticides and drugs that is also used in the production of pigments, sunscreens and fluorescent markers, and in the fragrance industry.

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Genome Research of Industrial Microorganisms

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The poster presentation summarizes activities of the group 'Genome Research of Industrial Microorganisms' carried out at the Center for Biotechnology (CeBiTec) at Bielefeld University.

Metagenomics and metatranscriptomics of biogas-producing and ensiling microbial communities is a major topic at the department. Corresponding projects involve characterization of biogas and ensiling communities by high-throughput 16S rDNA amplicon sequencing, metagenomics and metatranscriptomics to address the metabolically active community. These approaches aim at optimization of the biogas and ensiling process technologies.

Another focus of the group is the biological control of soil-borne fungal pathogens by bioeffector strains such as *Bacillus amyloliquefaciens*. Within the corresponding project, the complete genome sequence of an isolate representing the important fungal pathogen *Rhizoctonia solani* AG1-IB has been established. Moreover, the interaction of the biocontrol strain and the pathogen is studied at the molecular level by transcriptomics approaches.

The last topic involves genome analyses of antibiotic resistance and degradative plasmids from clinical and environmental bacteria. Dissemination of resistance determinants was followed between these habitats and detailed comparative genomic analyses of corresponding plasmids were undertaken. Plasmid metagenome studies addressed the characterization of the plasmid mobilome of bacteria residing in wastewater treatment habitats.

Structural Determinants of Aminomutases and Ammonia Lyases

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Beta-amino acids harbor many applications in their free form and as robust polymeric derivatives, such as beta-peptides [1]. Asymmetric syntheses of enantiomerically pure beta-amino acids and chiral amines have become a prevalent route using biocatalysts, such as aminotransferases, ammonia lyases, and aminomutases [2]. The aim of this work is to engineer a phenylalanine aminomutase (PAM) to acquire lyase properties for the efficient production of enantiopure beta-phenylalanine (a key component in the anti-cancer drug, taxol [3]).

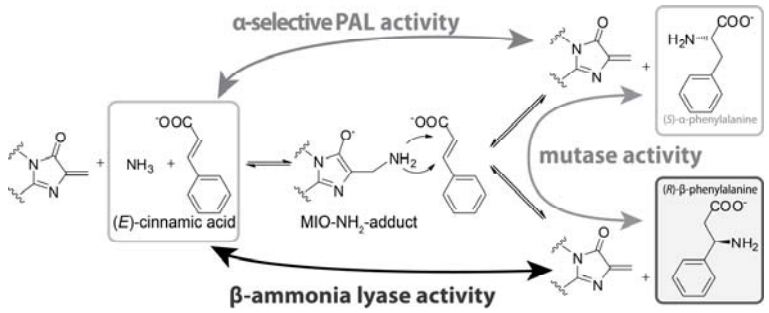

Fig. 1. Starting with an alpha-amino acid, PAM can either catalyze a mutase reaction producing beta-phenylalanine or a lyase reaction that yields cinnamate. The ratio between these reactiosn

was modified by structure-based mutagenesis.

Recent studies have implicated loop regions as key structural determinants between PAM and PAL [4,5]. Here, we report active-site loop residues of PAM that influence mutase/lyase activity along with structural data that indicate inner loop structural effects on lyase/mutase preferences. Overall, this work represents the first successful attempt to convert a PAM to a PAL through strict mutase-to-lyase residue mutations and may guide future engineering of faster PAM variants to be used for the efficient synthesis of beta-phenylalanine. These results partially explain functional determinants between aminomutases and ammonia lyases.

Acknowledgement: this work was supported by the EU-FP7 project (ENEFP –Grant 215560).

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Novel multi-species microbial consortia involved in lignocellulose and 5-hydroxymethylfurffural bioconversion

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To develop a targeted metagenomics approach for analysis of novel multi-species microbial consortia involved in the bioconversion of lignocellulose and furanic compounds, we applied replicated sequential-batch aerobic enrichment cultures with either pretreated or untreated wheat straw as the sources of carbon and energy. After each transfer, exponential growth of bacteria was detected using microscopic cell counts, indicating that the substrate was being utilized. In batch, the final bacterial abundances increased from an estimated 5 to 8.7-9.5 log 16S rRNA gene copy numbers/ml. The abundances of fungal propagules showed greater variation, i.e. between 5.4 and 8.0 log ITS1 copies/ml. Denaturing gradient gel electrophoresis analyses showed that the bacterial consortia in both treatments reached approximate structural stability after six transfers. Moreover, the structures of the fungal communities were strongly influenced by substrate treatment. A total of 124 bacterial strains were isolated from the two types of enrichment cultures. The most abundant strains were affiliated with the genera *Raoultella/Klebsiella*, *Kluyvera*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium* and *Arthrobacter*. Totals of 43 and 11 strains obtained from the untreated and pretreated substrates, respectively, showed (hemi)cellulolytic activity (CMC-ase and xylanase), whereas 96 strains were capable of growth in 7.5mM 5-hydroxymethylfurfural. About 50% of the latter showed extracellular oxidoreductase activity as detected by a novel iodide oxidation method. Also, (hemi)cellulolytic fungal strains related to *Coniochaeta*, Plectosphaerella and *Penicillium* were isolated. One *Trichosporon* strain was isolated from pretreated wheat straw. The analysis of bacterial succession and composition was achieved by 16S rRNA gene amplicon sequencing performed with both enriched cultures. Most taxa thriving in the lignocellulosic mixed cultures (or involved in bioconversion of furanic compounds) belonged to the orders Enterobacteriales, Pseudomonadales, Xanthomonadales, Flavobacteriales and Sphingomonadales orders. The two novel bacterial-fungal consortia are starting points for lignocellulose degradation applications.

Increasing Thermostability of Halohydrin Dehalogenase (HheC) by Computation

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For many industrial applications and laboratory purposes it is desirable to engineer enzymes towards enhanced thermostability. During the past decades, several approaches for protein thermostabilization have been developed, which however require time-consuming multiple rounds of mutagenesis and selection or yield only modest improvements in apparent melting temperature. In this study we used computational methods [1] according to the so-called FRESCO approach (Framework for Enzyme Stabilisation by Computation) to design small libraries which contain predicted thermostability-enhancing mutations of halohydrin dehalogenase (HheC) [2], and combined experimentally successful mutations to obtain highly thermostable multi-site mutants.

On the basis of the known HheC X-ray structure (1PX0), all possible disulfide-bonds and point mutations were designed in silico using DisulfideDiscovery, Rosetta and FoldX software and were ranked according to their $\Delta\Delta G^{fold}$. From this set, 47 disulfide bond mutants and 775 point mutants were evaluated by MD simulations and the results were visually inspected to eliminate (false positive) variants predicted to have increased disorder or flexibility. The remaining 35 disulfide bond mutants and 218 point mutants were selected for experimental testing. For this the mutants were constructed in microtiter plate format, expressed, purified and evaluated using a fluorescence assay.

The results demonstrated that although introduction of disulfide bonds hardly contributed to thermostability. On the other hand, 29 stabilizing point mutations were found with $\Delta T_m^{app} \geq 1°C$. Combining 13 of the most stabilizing point mutations resulted in a mutant with a 25.5°C increase in its thermal stability comparing to wild-type. Dehalogenase activity was retained. The thermostable mutant will be used as template for further engineering towards a favorable industrial biocatalyst.

The results show that the orthogonal computational methods of the FRESCO approach allow efficient enzyme thermostabilisation while minimizing experimental screening.

Acknowledgement: this work is supported by the FP7 Kyrobio project (Grant agreement no. 289646).

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Artificial enrichment of aromatic hydrocarbon in "Amazon Dark Earth" to explore ecological changes in bacterial catabolic gene *bph*

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"Amazon Dark Earth" (ADE) is a term used to describe horizons in Amazon soils. Due to high concentration of organic matter and black carbon, this soil became an important source to study genes related to the degradation of aromatic compounds. Generally, catabolic genes are studied in aromatic compounds contaminated soils, so, this study aimed to examine the effects of hydrocarbons addition in ADE and their adjacent (ADJ) soils (no anthropogenic soils). Microcosms were constructed in order to follow changes in total bacterial community structure (16S rRNA gene) and aromatic hydrocarbon degrader bacterial community structure (*bph* gene) through fingerprint technique (T-RFLP), and also to analyze these genes abundance through qPCR, in soil samples before and after hydrocarbons incubation. It was used soil samples of four Amazon sites: two ADE soil sites, and two ADJ soil sites, both under different land use (secondary forest: SF; agricultural cultivation: CULT). The T-RFLP results showed no changes in total bacterial community structure after incubation for all studied soils. However, it was observed a shift in community structure of *bph* gene and a increasing of operational taxonomic units (OTUs), for this gene, after incubation for all studied soils. Principal Component Analysis of T-RFLP profiles of 16S rRNA and *bph* genes communities' structure showed distinct groupings. In general it was observed clusters distinguishing ADE from ADJ soils (under FS and CULT) for 16S rRNA and *bph* genes. These clusters were observed for soils before and after treatment with hydrocarbons, which indicates that the structure of the studied genes was more influenced by the difference of anthropogenic soils in relation to their originals, than by the land use. The qPCR results reported a significant (Tukey, $p < 0.01$) increase in the number of copies of *bph* gene per gram of soil after hydrocarbons incubation for all soils. These results displayed the influence of aromatic compounds in the structure of aromatic hydrocarbon degrader bacterial community in ADE and ADJ soils. This study can shed some light on the role of catabolic genes in these soils and highlight the need to further study the factors controlling the diversity and function of aromatic compounds degrader bacteria groups in Amazonian soils.

Molecular tools for habitat selection and Metagenomics

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Our interest is in enzymes that allow the breakdown of natural sources of carbonaceous plant tissues, i.e. lignocellulose and hemicellulose. In particular, we focus on organisms carrying novel (hemi) cellulases next to laccases/ oxidative/ substrate-binding enzymes. Thus, natural microbial communities from different environments are explored to access the vast source of novel (new) enzymes via metagenomic studies. For this purpose, we develop and apply a novel horizontal gene transfer expression system, which allows to spread cloned fragments of metagenomic DNA across a wide variety of hosts. Thus, the novel HGT based multi-host/vector system is combined with growth selection and screening. This will allow a boost in our ability to detect/screen activities. To construct the novel vector system, we use the highly promiscuous and self-transferable plasmid pIPO2 (PromA plasmid) as the basis. The plasmid was dissected, and a multiple cloning site inserted. Positive selection for insertion is warranted by an active cytotoxic ccdB killer gene. Maximum insert sizes are expected to range up to 20-25 kb, allowing stability of the plasmid. Copy numbers of the plasmid are expected to allow relatively high levels of gene expression. The poster will report on several features of this novel metagenomic cloning system.

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