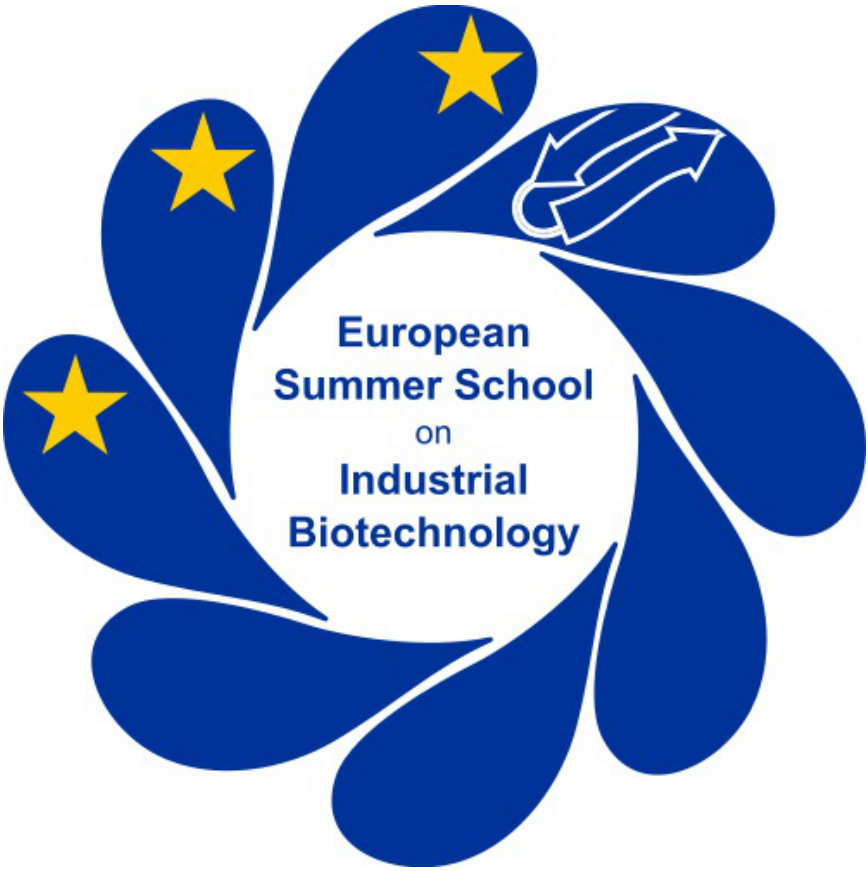

5th EUROPEAN SUMMER SCHOOL ON INDUSTRIAL BIOTECHNOLOGY

Functional metagenomics: from nature to biochemical functions

HAMBURG, JULY 3-6, 2023

The European Summer School on Industrial Biotechnology (ESSIB) is organized by the University of Milano-Bicocca (Department of Biotechnology and Biosciences) and, the University of Stuttgart (Institute of Biochemistry and Technical Biochemistry). ESSIB addresses PhD students and postdocs, preferentially from European laboratories, and will take place every year in a different country and with a different focus. ESSIB is a complete educational program based on lectures, practical laboratory experience, and contacts with companies active in the field.

The 5th ESSIB

Lectures

Rolf Daniel (University of Göttingen) „Metagenomics to explore and exploit the hidden microbial potential and diversity“
Pablo Pérez-García (University of Hamburg) „An in vitro and reporter-strain coupled pipeline for the discovery of plastic-degrading enzymes“
Andrew Pickford (University of Portsmouth) „Characterising plastic-degrading enzymes: from biophysics to bioreactors“
Jürgen Pleiss (University of Stuttgart) „Digitalization of metagenomics“
Manuel Ferrer (CSIC Madrid) „Best using metagenomic data to bring enzymes to industry and consumers“
Ruth Schmitz (University of Kiel) „Interbacterial relationships: From biofilms to jellyfish“
Erik Borchert (Geomar, Kiel) „Hidden ecological functions of the marine Plastisphere“
Peter Golyshin (University of Bangor) „Polyester-degrading enzymes from microorganisms inhabiting hot vents“
Federica Bertocchini (CSIC, Madrid) „Plastic degradation by wax worm Peases“
Ren Wei (University of Greifswald) „Mechanism-Guided Discovery and Engineering of PET Hydrolases“
Victor Guallar (University of Barcelona) „What's next? AI modelling in enzyme bioprospecting and engineering“
Stephan Kolkenbrock (Altona Diagnostics, Hamburg) „Mining Metagenomes for RNA- and DNA-directed DNA Polymerases“
Karl-Erich Jaeger (University of Düsseldorf) „Bacterial chassis for monitoring and production of valuable compounds“
Rainhard Koch (formerly Bayer) „Use of enzymes in applied research“

Major Topics

Finding genes and enzymes
Mining the sea
Finding plastic-active enzymes & others
From (meta) genomes to applications

Practical courses

Cell-free expression
Screening activities of novel enzymes
Computational tools for metagenomic enzyme search

Exciting excursion

To European XFEL – The world's largest X-ray laser

Dear participants,
We welcome you and thank very much all contributors. Have a good time!

Wolfgang Streit, Marina Lotti, Jürgen Pleiss & the ESSIB organizing team

Program

Time	Monday, July 3: Finding genes and enzymes
08:30	Registration
09:00	Welcome and Introduction (Wolfgang Streit/Marina Lotti/ Jürgen Pleiss)
09:15	**Talk 1 Rolf Daniel** (Göttingen) “Metagenomics to explore and exploit the hidden microbial potential and diversity”
10:00	**Talk 2 Pablo Pérez-García** (Hamburg/Kiel) “An in vitro and reporter-strain coupled pipeline for the discovery of plastic-degrading enzymes”
10:45	Coffee Break
11:00	**Talk 3 Andrew Pickford** (Portsmouth) “Characterizing plastic-degrading enzymes: from biophysics to bioreactors”
11:45	**Talk 4 Jürgen Pleiss** (Stuttgart) "Digitalization of metagenomics"
12:30	Lunch break (Mensa)
13:30	**Participants´ intro:** 3 min presentations by students
15:00	**Lab course:** Hands-on Metagenomics
18:30	Beer/Pretzel and sundowner at terrace of botanical garden

Time	Tuesday, July 4: Mining the sea
09:00	Arrival and organizational matters
09:15	**Talk 5 Manuel Ferrer** (CSIC Madrid) “Best using meta-genomic data to bring enzymes to industry and consumers”
10:00	**Talk 6 Ruth Schmitz** (Kiel) “Interbacterial relationships: From biofilms to jellyfish”
10:45	Coffee Break
11:00	**Talk 7 Erik Borchert** (Geomar, Kiel) “Hidden ecological functions of the marine Plastisphere”
11:45	Lunch break (Mensa)
12:45	**Poster session**
14:15	**Lab course:** Hands-on Metagenomics / computational tools
19:30	Beer at Hofbräu Wirtshaus Esplanade

Your notes

Program

Time	Wednesday, July 5: Finding plastic-active enzymes and others
09:00	Arrival and organizational matters
09:15	**Talk 8 Peter Golyshin** (Bangor) “Polyester-degrading enzymes from microorganisms inhabiting hot vents”
10:00	**Talk 9 Federica Bertocchini** (CSIC, Madrid) “Plastic degradation by wax worm PEases”
10:45	Coffee Break
11:00	**Talk 10 Ren Wei** (Greifswald) “Mechanism-Guided Discovery and Engineering of PET Hydrolases”
11:45	Lunch break (Mensa)
13:00	**Excursion to European XFEL**
18:30	Workshop Dinner at “Zm Alten Lotsenhaus”/Övelgönne

Time	Thursday, July 6: From (meta) genomes to applications
09:00	**Talk 11 Victor Guallar** (Barcelona) “What's next? AI modelling in enzyme bioprospecting and engineering”
09:30	**Talk 12 Stephan Kolkenbrock** (Altona Diagnostics) “Mining Metagenomes for RNA- and DNA-directed DNA Polymerases”
10:00	Coffee Break
10:15	**Talk 13 Karl-Erich Jaeger** (Düsseldorf/Jülich) “Bacterial chassis for monitoring and production of valuable compounds”
10:45	**Talk 14 Rainhard Koch** (formerly Bayer) “Use of enzymes in applied research”
11:15	Final discussion & farewell for ESSIB participants
12:00	Lunch (Mensa)

Your notes

Dr. Stefan Thiele Poster #5
University of Bergen, Norway

Current research topics: Arctic microbiology (marine, sea ice, permafrost), microplastic

Seasonality of the bacterial and archaeal community composition of the Northern Barents Sea

Background: The Barents Sea is a transition zone between the Atlantic and the Arctic Ocean and is particularly vulnerable due to the high variability of the ecosystem, especially with respect to sea ice coverage. With global warming and retreating sea ice, also the Northern Barents Sea is becoming accessible. Objectives: In order to improve our understanding of the pelagic marine microbial ecosystem in this area we investigated the bacterial and archaeal communities in different seasons (early and late winter, spring, and summer) as well as in different years (summers 2018, 2019, and 2021) along a transect through the Barents Sea into the Arctic Ocean. Methods: We used 16S rRNA gene sequencing for community analyses, and inferred functions from a genome database using PiCRUST2. Results: Winter samples were dominated by members of the SAR11 clade and a community of nitrifiers, including Cand. Nitrosopumilus and LS-NOB (Nitrospinia). During spring and summer successions of different members of the Gammaproteobacteria and Bacteroidia were seen, based on their utilization of different phytoplankton derived carbon sources but varied over the years with respect to sea ice coverage. This indicates that Arctic marine microbial ecosystems in this region switch from carbon cycling in spring and summer to nitrogen cycling in winter. Due to global warming, these nutrient cycles might change with unknown consequences for the ecosystem of the Barents Sea.

Dr. Carolina Ilenia Giunta & Emilio Cutrona Poster #6 #7
INOFEA AG, Switzerland

Current research topics: Enzyme immobilization for FuturEnzyme

Enhancing enzymes properties for industrial application: how to solve the challenge

Owning excellent catalytic properties, enzymes are turnkey solutions for several industrial and academic applications. However, their poor stability in unfavorable conditions represents a major limit in industrial processes. A consistent need of enhancing the properties of such natural biocatalysts exists. The challenge is to allow an easier and faster implementation of the enzymes in a broad range of industrial applications. The enzzen® technology besides enhancing the stability of enzymes of different classes, confers new properties. The technology is based on the chemical and physical tuning of the enzyme environment at a nanometer scale. Herein, we showcase different examples of applications of the enzzen® technology platform where the biocatalysts were stabilized in acidic pH environment, high temperatures and in presence of organic solvents. Moreover, examples of non-enantioselective esterases and lipases turned into fully enantioselective enzymes are displayed, showing the potential of such biotechnological solution. These findings open the opportunity to implement greener and more sustainable industrial manufacturing.

Dr. Jessica Zampolli Poster #8
University of Milano-Bicocca, Italy

Current research topics: Environmental microbiology - Microbial plastic degradation

Microbial degradation of plastics: From a transcriptomic approach towards biodegradative functions of Rhodococcus opacus R7 grown on polyethylene

Zampolli J.[1]*, Vezzini, D.[1], Orro A.[2], Natalello A.[1], Ami D.[1], Mangiagalli, M.[1], Lotti, M.[1], Di Gennaro P. [1] [1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy [2] Institute of Biomedical Technologies, National Research Council, CNR, ITB-CNR, Italy

Plastic waste management has become a global issue, and among other plastics, polyethylene (PE) is the most abundant synthetic plastic worldwide, and low-density PE generates the largest volume of plastic pollution. Although PE is one of the most resistant to biodegradation for its properties, few bacteria can directly use PE as the sole carbon and energy source without any physical or chemical pretreatments. In this context, an omic-approach contributed to unraveling the complex degradative system behind PE biodegradation to clarify the gene framework for PE biodegradation of Rhodococcus opacus R7. This strain was selected for its ability to grow on PE as the only carbon and energy source in a short range of time. RNA-seq uncovered genes putatively involved in the first step of PE oxidation. In-depth investigations through bioinformatic analyses and enzymatic assays on the supernatant of R7 grown in the presence of PE confirmed the activation of superficial or releasing extracellular enzymes genes encoding for laccase-like enzymes. Moreover, the transcriptomic data showed candidate genes for the subsequent steps of short aliphatic chain oxidation including alkB gene encoding an alkane monooxygenase, cyp450 gene encoding cytochrome P450 hydroxylase, and genes encoding membrane transporters. PE biodegradative system was also validated by FTIR analysis on R7 cells grown on PE compared to malate condition indicating that PE induces metabolic changes, including longer lipid hydrocarbon chains in membranes, and a more intense beta-sheet band. These results pose the basis for environmental and biotechnological applications contributing to plastic elimination.

Rubén Muñoz Tafalla Poster #9
Barcelona Supercomputing Center, Spain

Current research topics: Enzyme engineering, Computational Enzyme Bioprospecting

Computational Bioprospecting of a Bacterial Lipase

Nowadays, a vast amount of proteins are annotated in protein databases every day, without even knowing their specific role, let alone non-natural and human-profitable functions. This work aims to find, through computational bioprospecting, new enzymes that could outperform the current ones used in triglyceride degradation. To achieve this, we developed a hierarchical workflow based on searching sequences, filtering them, and running protein-ligand simulations. At first, a PSI-Blast search of sequences was done using a lipase discovered in the FuturEnzyme European Consortium as a seed sequence (FELip9). Through this methodology, 525 sequences were found. After downloading the Alphafold structures and filtering by sequence and structure, a total of 288 esterases were simulated with 15 ligands, which were different size triglycerides, using PELE. PELE (Protein Energy Landscape Exploration) is an in-house all-atom Monte Carlo molecular modeling sampling software used in this project to calculate the affinity of the ligands to the enzymes active sites. PELE results can be used to rank the best-performing enzymes against a single triglyceride but also against grease stains, which consist of many different triglycerides, as we can select the best enzymes for several ligands. This work is included in work package 2 of the FuturEnzyme project, and the discovered enzymes will be used in the following work packages.

Agnieszka Maciejewska Poster #11
altona Diagnostics GmbH, Germany

Current research topics: Molecular Bioengineering, Nanotechnology, Enzymology

Conditional reactivation of enzyme-catalyzed reactions

Enzymes play a pivotal role in biotechnology, molecular biology, and many other fields of science. Their activity is crucial for catalyzing reactions within all living organisms as well as in industrial applications. To obtain the highest efficiency and best control over a reaction, it is desired that the enzyme is active precisely at the time when its function is required. For example, in a reverse transcription-polymerase chain reaction (RT-PCR) two enzymes drive the polymerization reaction. The reverse transcriptase (RT), which is facilitating the synthesis of a cDNA strand from RNA templates, and the Taq DNA-polymerase, which replicates DNA strands. Activity of either of these enzymes before the start of the desired reaction can lead to decreased specificity and sensitivity of the RT-PCR. To control activity of the Taq DNA-polymerase there are well established solutions involving the use of e.g., antibodies or aptamers. However due to the difference in thermal stability between the Taq DNA-polymerase and RT, suitable solutions for the latter still need further development. The aim of the project is to find generalizable methods for enzyme inactivation and conditional reactivation by separating it from other reaction components. Particular focus is paid to the possibilities of nano-formulations. The enzymes could be genetically modified to allow specific design and properties enabling their effective separation. As part of the project the respective methods to characterize the efficiency of immobilization and determine enzymatic activity will be developed. Investigating different approaches of separation of enzymes and reaction mix components will increase the understanding about the interactions between complex master

mixes and nanoparticles. Such a platform could be adjusted to use with other components needed in the PCR such as internal or positive controls, as well as with other enzymes for which the conditional reactivation is of great importance.

Melissa Bisaccia Poster #13
University of Insubria, Italy

Current research topics: Metagenomic community profiling and bioprospecting of extremophilic microorganisms from the Antarctic Ocean for the production of industrially-relevant enzymes (e.g., laccases and chitin deacetylases) and specialized metabolites.

A novel promising laccase from the psychrotolerant and halotolerant Antarctic marine Halomonas sp. M68 strain

Melissa Bisaccia[1], Elisa Binda[1], Elena Rosini[1], Gabriella Caruso[2], Ombretta Dell'Acqua[3], Maurizio Azzaro[2], Pasqualina Laganà[4], Gabriella Tedeschi[5,6], Elisa M. Maffioli[5,6], Loredano Pollegioni[1] and Flavia Marinelli[1] [1] Department of Biotechnology and Life Sciences (DBSV), University of Insubria, Varese, Italy, [2] National Research Council, Institute of Polar Sciences (CNR-ISP), Messina, Italy, [3] National Research Council, Institute of Polar Sciences (CNR-ISP), Venice, Italy, [4] Department of Biomedical and Dental Sciences and Morphofunctional Imaging (BIOMORF), University of Messina, Messina, Italy, [5] Department of Veterinary Medicine and Animal Science (DIVAS), University of Milan, Milan, Italy, [6] Cimaina, University of Milan, Milan, Italy

Microbial communities inhabiting the Antarctic Ocean show psychrophilic and halophilic adaptations conferring interesting properties to the enzymes they produce, which could be exploited in biotechnology and bioremediation processes. Use of cold- and salt-tolerant enzymes allows to limit costs, reduce contaminations, and minimize pretreatment steps. In the frame of the PNRA16_00105 ANT-Biofilm Project, we screened 186 morphologically diverse microorganisms isolated from marine biofilm and water samples collected in Terra Nova Bay (Ross Sea, Antarctica) for the identification of several types of hydrolytic and oxidative enzymatic activities, with a particular focus toward new laccase activities. After primary screening, 13.4% and 10.8% of the isolates were identified for the ability to oxidize 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the dye azure B, respectively. Amongst them, the marine Halomonas sp. strain M68

showed the highest activity. Production of its laccase-like activity increased six-fold when copper was added to culture medium. Enzymatic activity guided separation coupled with mass spectrometry identified this intracellular laccase-like protein (named Ant laccase) as belonging to the copper resistance system multicopper oxidase family. Ant laccase oxidized ABTS and 2,6-dimethoxy phenol, working better at acid pHs. The enzyme showed a good thermostability, with optimal temperature in the 40–50°C range and maintaining more than 40% of its maximal activity even at 10°C. Furthermore, Ant laccase was salt- and organic solvent-tolerant, paving the way for its use in harsh conditions. Overall, the features of Ant laccase make it a promising candidate for biotechnological and industrial use.

Luca Mellere Poster #15
University of Insubria, Italy

Current research topics: Microbial Biotechnology

Exploring Coriolopsis gallica ligninolytic system for the discovery of industrially relevant laccases

Coriolopsis gallica is a basidiomycete fungus which utilizes a system of lignin-modifying enzymes in order to colonize and decompose dead wood. Its extracellular oxidative system includes laccases, polyphenol oxidases able to oxidase a wide range of compounds making them suitable for different industrial applications. The aim of this work was to explore the production of laccases in C. gallica MUT00003379 and to improve the industrial application of white-rot fungi as cell factories for the production of lignin-modifying enzymes. Through a screening of different media, supplemented with copper sulphate, a production process was successfully developed leading to a yield of 200 000 U L-1. Both NATIVE and SDS-PAGE analysis revealed the presence of a single 60 KDa secreted protein with laccase activity. Initial characterization of the enzyme revealed maximum activity on 2,2-Azino-bis-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) at pH 4 in sodium acetate buffer. Optimal temperature was found to be around 60°C. The enzyme also resulted stable for more than 1440 hours in alkaline buffers maintaining a relative activity of 60-70%. Further characterization of the enzyme activity on different substrates and the relative kinetic parameters is currently in progress. Moreover, solid-state fermentation processes are currently being developed to further explore the diversity of the laccases produced by the fungal strain in order to discover new industrially relevant enzymes and applications.

Filippo Molinari Poster #16
University of Insubria, Italy

Current research topics: Industrial biotechnology, Biochemistry, Strain engineering, Microbial bioconversion

Sustainable production of cis,cis-muconic acid: a whole-cell bioconversion of renewable biomasses-related aromatics

Filippo Molinari, Loredano Pollegioni and Elena Rosini Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy. e-mail: fmolinari@uninsubria.it cis,cis-Muconic acid (ccMA) is a valuable precursor for the synthesis of plastic materials, traditionally obtained from petroleum-based feedstock using high concentration of heavy metal catalysts. The sustainability of this production process is very low due to the generation of pollutant by-products and the requirement of downstream purification of the obtained mixture of cis,cis- and cis,trans-muconic acid. [1] To solve this issue, we developed an efficient and green process to produce ccMA from renewable feedstocks (i.e. Kraft lignin and wheat bran) based on: a) the optimization of the extraction procedures of vanillin from lignin and of ferulic acid from wheat bran; b) the genetic engineering of an E. coli strain to modulate the expression of up to seven recombinant enzymes. In details, vanillin was recovered from Kraft lignin by an enzymatic treatment using the recombinant Bacillus licheniformis laccase, [2] and ferulic acid from the wheat bran by a thermo-enzymatic method using the Ultraflo®XL commercial enzyme. The whole-cell biocatalyst to convert vanillin into ccMA expresses the dehydrogenase LigV, the demethylase VanAB, the decarboxylase AroY and the dioxygenase C12O; meanwhile the whole-cell biocatalyst to convert ferulic acid to ccMA expresses all the above-mentioned enzymes plus the decarboxylase Fdc and the dioxygenase Ado. The engineered strains converted >95% of lignin-derived vanillin in 30 minutes, obtaining the production of 4.2 mg ccMA/g of Kraft lignin. Starting from the wheat bran-derived ferulic acid, ccMA was produced with a >95% conversion yield in 10 hours, corresponding to 0.73 g ccMA/g ferulic acid, and 2.2 mg ccMA/g wheat bran biomass. These whole-cell systems, coupled with the extraction of vanillin and ferulic acid from renewable biomasses, could represent a sustainable alternative for the production of ccMA. [3] [1] Khalil I. et al., Green Chem. 2020, 22 (5), 1517–1541 [2] Vignali E. et al., ChemSusChem 2022, 15 (20), e202201147 [3] Molinari F. et al., ACS Sustain. Chem. Eng. 2022, 11 (6), 2476–2485.

Aurélien Lalou Poster #17
Fachhochschule Nordwestschweiz (FHNW), Switzerland

Current research topics: Genetic and functional interaction in bioremediation pathways

Protein-Protein Interaction in enzyme clusters

Genes clusters have been found to regroup enzymes belonging to particular pollutants degradation pathways. Deeper understanding of this clustering and its consequences on the genes products could yield interesting knowledge for bioremediation purposes. Are members of those clusters interacting with one another and can we highlight some sort of metabolic channeling?

Tobias Horbach Poster #18
Heinrich-Heine-Universität Düsseldorf, Germany

Current research topics: Metagenomics, enzyme assays, enrichment cultures from environmental samples

Activity- and sequence-based screening for polyester-degrading enzymes from Deep-Sea sediments

The annual increase in the amount of plastic waste confronts today's society with a steadily growing problem. The use of polymer-active enzymes represents a promising solution for breaking down polymers into their individual components. For this purpose, the focus was placed specifically on polyesterases from marine organisms that are found in plastic-polluted habitats and can specialize in the degradation of plastics. For this purpose, organisms were enriched for polyester-polyurethane starting from sediment samples from the deep sea of the North Atlantic and several marine strains were isolated that exhibited polyesterase activity. Analysis via partial 16S rDNA sequencing showed that these were predominantly unknown strains. Using hidden Markov models from known polyesterases, novel potential polyesterases were identified in the genomes associated with the isolates and nine genes were cloned. Initial testing of corresponding expression strains on detection plates showed active esterase expression in five strains, three of which exhibited polyesterase activity. Thus, three new polyester hydrolases from Pseudomonas sp. and Rhodococcus sp. are available for further characterization. Thus, new insights into the diversity of polyester-degrading enzymes of marine organisms could be provided, creating a new starting point for the screening and characterization of polyesterases. This leads to a better understanding of the still poorly studied class of polyesterases.

Francesca Demaria Poster #19
Fachhochschule Nordwestschweiz (FHNW), Switzerland

Current research topics: Microbiome dynamics on bioremediation of pharmaceuticals

Analysing microbial community dynamics and pharmaceuticals degradation in lab-scale MBRs

Pharmaceutical are emerging contaminants (ECs), which are in some cases recalcitrant to biodegradation. The release of these biologically active compounds into the environment causes adverse effects on ecosystems and living organisms. As bio-based solution, specific microbial communities have been identified as being able to degrade these persistent compounds and are representing a promising approach for a more sustainable removal of these compounds in wastewater. By using analytical methods combined with omic-technologies, we aim to ascertain microbial dynamisms and identify genes and enzyme involved in the degradation of pharmaceutical to improve their degradation.

Marcella de Divitiis Poster #20
University of Milan-Bicocca, Italy

Current research topics: Environmental Microbiology

Natural rubber degrading microbial communities: metagenomic assessment of the microbial diversity in the presence of rubber compounds

Natural rubber is a natural product, derived from Hevea Brasiliensis latex, and the main elastomer used in tires and rubber goods. Many studies in literature report the isolation of microbial strains capable of rubber degradation, but little is known about the consortia able to lead such biodegradation. New approaches, such as metagenomics, have become a powerful tool to investigate the biodiversity of soil microbial communities. The majority of soil microorganisms are still uncultured, many are uncultivable, and therefore culture-independent approaches are used to investigate the soil microbial communities by reconstructing hypothetical metabolic and signaling pathways, with the aim of identifying and assessing the activity, function, diversity, and evolution of soil microorganisms. Hence, our work aims to study the features of microbial communities developed in the presence of rubber compounds by understanding the structural and functional diversity present in rubber-contaminated soil with a metagenomics approach. Amplification of marker genes with high-throughput sequencing techniques was performed for analyzing composition, richness, and biodiversity of soil bacteria in rubber-polluted soil conditions. A subsequent bioinformatics analysis through the QIIME software provided a taxonomic classification and clustering of sequences in OTUs (operational taxonomic units). To analyze soil bacterial diversity, the alpha diversity (Shannon index) was calculated by QIIME for each sample and, to compare the diversity among communities, beta diversity was calculated using Bray Curtis (B-C) distance considering species' presence/absence and

relative abundances. Through this approach, a picture of the microbial communities developed nearby rubber materials will be provided. Starting from this, studies of the community's rubber degrading activities will be deepened in order to better understand the mechanisms of rubber biodegradation and exploiting them for bioremediation processes of contaminated sites.

Dr. Cristina Coscolín, Laura Fernández López & Paula Vidal Ramón
Institute of Catalysis – CSIC, Spain **Poster #22 #23 #24**

Current research topics: Biocatalysis, enzyme engineering

SysBio - Enzymes to the people: mitigate together climate change and promote circular bioeconomy

Laura Fernandez-Lopez[1], Paula Vidal[1], David Almendral[1], Patricia Molina-Espeja[1], Cristina Coscolín[1], Manuel Ferrer[1] [1]Instituto de Catalisis y Petroleoquimica, ICP, CSIC, Marie Curie 2, 28049, Madrid, Spain The Systems Biotechnology (SysBio) group (https://sysbio.csic.es/) is located at the Institute of Catalysis and Petrochemistry (ICP) (Madrid, Spain), which belongs to the Spanish National Research Council (CSIC).

Our scientific activity is marked into the biotechnology field, which uses microorganisms or parts of them to substitute traditional chemical reactions for greener and more efficient biological solutions. Our team is composed by chemists, biochemists and biologists which confer a multi- and inter-disciplinary view to all projects. The SysBio group offers and covers all steps from enzyme discovery (including meta-genomics) to engineering and implementation in multiple processes and products of interest for multiple industrial sectors. Subsequently, the accumulated knowledge of the bioprospecting and study of hundreds of new enzymes combined with engineering and material science techniques allowed the team to undertake leading investigations aimed at achieving a detailed understanding of the mechanisms underlying and tuning the properties of enzymes. Serve as examples recent works that investigate the mechanisms by which enzymes become promiscuous1, the adaptation of enzymes and the microbes that contain them to climate change2, and a recent investigation proposing a new strategy of enzyme supramolecular engineering based on a meticulous modification of a synthetic shell that surrounds an immobilized enzyme3. Most recently, the team members acquired expertise in computational techniques which have constituted the basis of a novel research addressing the

design of a new generation of artificial enzymes with multiple active sites, the PluriZymes4,5. Bioinformatics and computational integration of multiple enzyme reactivity data is also allowing us to contribute to establishing the basis for predicting enzyme properties from enzyme sequence without the need for enzyme characterization6. To undertake all these activities, in the context of our effort to circular economy and climate change fighting7, it is crucial to know which are the requirements of industry. This is why we are constantly working with several companies in our different projects from a wide variety of sectors, such as those covering fine chemicals, food and feed, cosmetics, textiles, detergents, etc. Companies give us a set of necessities and real materials to start with, which constitute the base for bioprospecting and engineering enzymes a-la-carte. Science popularization and gender equality are also driven the SysBio group, and all our projects have an ecofriendly orientation. In this contribution, we summed up relevant investigations driven the SysBio commitment for the implementation of biocatalysts in our everyday life so we can help to mitigate climate change and move towards bioeconomy and circular economy7.

References 1Martínez-Martínez M, et al. ACS Chem Biol. 2018; 13:225-234. 2Marasco R, et al. Nat Commun. 2023; 14:1045. 3Giunta CI, et al. ACS Nano. 2020; 14:17652-17664. 4Alonso S, et al. Nat Catal 2020; 3:319-328. 5Roda S, et al. Angew Chem Int Ed Engl. 2022; 61:e202207344. 6Xiang R, et al. Biomolecules. 2022; 12:1529. 7Molina-Espeja P, et al. Oxford Open Climate Change, 2023; kgad003. Acknowledgements We acknowledge the FuturEnzyme Project funded by the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No. 101000327), and the Grants PID2020-112758RB-I00, PDC2021-121534-I00, and TED2021-130544B-I00 from the Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación (AEI) (Digital Object Identifier 10.13039/501100011033), and the European Union ("NextGenerationEU/PRTR").

Dr. Alberto Baeri Poster #25
University of Milano-Bicocca, Italy

Current research topics: Mucosal Immunology, iNKT, IBD, Fibrosis, Gut Microbiota

Elaboration of an in vitro test for the carcinogenesis evaluation of chemical and natural compounds used in the cosmetic industry

Toxicological tests for cosmetic products are classically performed on animal models. Prohibitive costs and evolution of the perception about animal experimentation in the general public have encouraged the development of in vitro tests capable of predicting the toxicity of compounds potentially classifiable as "CMR" (Carcinogenic, Mutagenic and/or Reprotoxic). In this work, we establish relevant transcriptomic signatures of CMR compounds on lung epithelia using 2D (such as the normal human bronchial epithelium cells, BEA-2B) and 3D cultures. 3D cultures defined as "air liquid interface" (ALI) reconstitute a differentiated epithelium composed of different types of cells (ciliated cells, goblet cells, etc.). Two known CMR toxicants (cadmium chloride (CdCl2), hydroquinone (HQ)) were selected according to previous literature. ALI and BEAS-2B cultures were first analysed by microarray gene expression profiling upon incubation with the two toxicants. This transcriptomic analysis performed on bulk cells revealed a comparable response based on a 200 genes signature between the two culture systems used and a better reproducibility when using the BEAS-2B model. Next, we performed single cell analysis to identify potential bias linked to the ALI culture systems and differences in the biological response to the treatment at the cell subpopulations level. We identified cell-type specific responses that allowed us to establish a transcriptomic signature for each cell type composing the ALI system in response to the toxicants and a hierarchy of "responding cell types". Overall, our results show that

the ALI system associated with a Single cell analysis can be successful used as an alternative to in vivo inhalation toxicology studies.

Miguel Luengo Perez Poster #26
Barcelona Supercomputing Center, Spain

Current research topics: Enzyme Engineering

Designing a new generation of industrial proteases

Enzymes are essential in many industrial biotechnological processes ranging from detergents and cosmetics to the food industry. Among the most relevant enzyme groups are proteases, accounting for 60% of the entire enzyme market. They are one of the main ingredients in detergent formulations, and their use accounts for approximately 30% of the total worldwide sales of enzymes. Considering their commercial impact, there has been a great interest in obtaining highly optimized proteases in the detergent industry. However, the elevated cost of production is still a hurdle in their industrial exploitation. One way to address this is by improving the activity and promiscuity of the enzymes. Increasing the former makes the reaction more efficient, reducing the amount of enzyme required, while increasing the latter means that a single enzyme variant can eliminate a more comprehensive range of organic stains. An interesting approach for improving an enzyme's overall promiscuity and activity is to design secondary active sites with a substrate specificity complementary to the primary one. However, almost all industrial subtilisin detergent proteases have few cavities besides the native one, which poses a challenge for designing additional catalytic pockets. Here, we present a novel enzyme design protocol that attempts to place new protease sites by searching the protein surface to insert extra catalytic triads. By performing a Montecarlo search of the protein-ligand complex mutations are designed to simultaneously improve the triad stabilization and the substrate binding affinity. These mutations create a binding complementary surface in the protein crevices where the ligand can be bound in a catalytically competent manner.

Andrea Rodriguez Sanz Poster #27
Universidade de Vigo, Spain

Current research topics: Obtention of functional compounds from agricultural waste and other material by enzymatic technologies

Algal microbiote as a potential source for new enzymes for seaweed biorefinery

Rodríguez-Sanz, A., Luaces A.,, Fuciños C., Torrado, A., Rúa M.L. Macroalgal surfaces provide a suitable substratum for the attachment of microbial colonizers, including fungi and bacteria, with densities reaching levels ranging from 102 to 107 cells cm-2 (1).

Some algal species may associate complex bacterial communities that are influenced by the composition of the algal surfaces and its exudates (2). In addition to specific seasonal and temporal variations, the physiological state of macroalgae also seems to influence the structure of algal associated microbial communities such as those occurring during the natural or forced decaying of the algae. By using metagenomic approaches, mainly based on 16S ribosomal RNA gene sequences, many novel species and genera have been identified in decaying algae-associated microbiota. Using culture-dependent approaches other authors have also isolated and identified several algal-polysaccharide-degrading bacteria (some representing new species) from the microbiota associated with the brown alga A. nodosum (3), which displayed multiple hydrolytic enzymes including hydroxyethyl cellulase, lichenase, and pectinase activities (4). All these studies suggest that there is still a wide field of research that should be explored for the search for new hydrolytic enzymes. They could play a main role as green tools in an algae biorefiney framework for developing enzyme-assisted extraction strategies that will allow improving the yields of extraction of algal components with cosmeceutical, functional food, nutraceutical, and biopharmaceutical applications in a sustainable and clean way (4). This investigation is a first approach to the evaluation of the bacterial diversity that colonizes the surface of fresh and decaying wild red algae with the further objective of identifying new carbohydrolases to be used in the development of a biorefinery scheme for red algae valorisation. Applying metagenomics techniques based on the 16S RNA it was possible to identify remarkably variations in the relative abundance between fresh and decaying algae. Bacteria belonging to the genera Flavobacterium, Shewanella and Pseudomonas were representative in the surface of decaying red algae, in contrast with fresh algae, where their relative abundance was less than 2%. Microorganisms included in these genera have already been described as carbohydrolases producers, specifically for carrageenan hydrolase (5), fucoidanase (6), alginate lyase (7) and β-agarose (8), β-1,3-xylanases (9, 10).

Ana Robles Martín Poster #28
Barcelona Supercomputing Center, Spain

Current research topics:

Designing Mutations for Improved Substrate Binding Affinities in Lipases

Lipases are essential enzymes to the detergent industry because of their efficacy in removing stains and odors from fabrics while also being environmentally friendly alternatives: they are capable of breaking down complex molecules, require lower temperatures, and are biodegradable. Enhancing the substrate binding affinity of lipases is crucial for improving their efficiency and reducing detergent usage, leading to energy savings. To achieve this, we conducted a study utilizing computational protein engineering to design and optimize the function of triacylglycerol lipases (EC. 3.1.1.3). We used a software program called AsiteDesign, which integrates PyRosetta modules with advanced sampling techniques to generate and evaluate several mutations with increased substrate binding affinities. The mutations we designed aimed to optimize existing binding pockets and create new ones, resulting in more efficient enzyme-substrate interactions. We assessed the effectiveness of these designs using PELE software, which employs a Monte Carlo local exploration of the active site with different triglyceride substrates to create interaction energy landscapes that can serve as proxies for enzymatic activity. Our study suggests that this approach can enhance the efficiency of detergent lipases, reducing environmental impact and increasing economic benefits for the industry. Future research will focus on evaluating the efficacy of these mutants through in vitro experiments.

Laura Marturano Poster #29
CNR-Institute of Polar Sciences (ISP), Italy

Current research topics: Extremophilic microorganisms

Extremophilic microorganisms as a resource for new enzymes for green applications

The commercial production of enzymes has increased over the past century in response to the many market applications in which they are involved, including but not limited to detergents, textiles, chemicals, and food. By "bioprospecting" we mean the study of living beings and biological materials in search of biomolecules that can be useful to humans. Extremophilic microorganisms such as psychrophiles, halophiles, and thermophiles are untapped and poorly described genetic resources with great potential to discover new and valuable enzymes suitable for biotechnological applications. About a hundred microorganisms, including bacteria and archaea, isolated from various ecosystems (deep sea water and sediments, shallow hydrothermal vents, Antarctic environments, hypersaline areas) were selected and analyzed for useful and applicable enzymatic activity. Screening was carried out using qualitative enzymatic assays based on the degradation of substances such as various lipids, milk proteins and hyaluronic acid. As a result, a group of prokaryotic (bacteria and archaea) strains with innovative properties was identified. In particular, approximately half of the isolates tested were positive for lipase, 15% positive for hydrolase, and 10% positive for hyaluronidase. The most promising HA-depolimerizing bacteria were two Vibrio sp. and one Paracoccus sp. isolates. Further genomic analyzes will be carried out to better understand the molecular processes to increase the production of new enzymes.

Cristina Palacios-Mateo Poster #30
Maastricht University, Netherlands

Current research topics: Enzymatic de-polymerization of polyester microfibers

Enzymatic de-polymerization of polyester microfibers in water, soil and sewage sludge as bioremediation strategy against pollution in agricultural fields

Microfibers have been found everywhere on the planet, one known source of microfibers being domestic laundering. In waste water treatment plants, large microfibers end up in the sewage sludge, while a significant amount of smaller microfibers remain in the effluent water. Sewage sludge, along with compost and manure, are commonly used as biofertilizers in agriculture, as they help to increase crop yields. However, there is increasing evidence that these practices significantly contribute to microplastic contamination in agricultural soils. As a result, microfibers have been found in edible fruits and vegetables. With this poster, we want to draw attention to the use of enzymes (and specifically hydrolases) as potential solution to either recycle or degrade microfibers. We envision three intervention points where hydrolases could be used: 1) enzymatic recycling of microfibers captured by laundry filters, 2) enzymatic treatment of biofertilizers and irrigation water, and 3) in situ enzymatic bioremediation of soil. In order to prove the feasibility of this proposal, enzymatic de-polymerization tests on polyester microfibers were performed in vitro in different mediums and temperatures. The chosen enzymes were leaf-branch compost cutinase (LCC) and FAST-PETase. Our results (analyzed by HPLC, FTIR, GPC and DSC) successfully show degradation of the polyester substrates, proving the potential of this technology in the fight against microfibers.

Andrea Salini Poster #31
Università degli Studi di Verona, Italy

Current research topics: Plastic-degradation, enzyme discovery

Exploring the plastic-degrading potential of microbial consortia isolated from a local wastewater treatment plant

World plastic production has promptly grown over the last decades reaching a global production of more than 350 million tons in 2020. Poor waste management practices pose a threat to the environment as plastic waste accumulates in several ecosystems and decays into microplastics. Moreover, a significant amount of microplastics leak into the environment due to incomplete removal in wastewater treatment plants (WWTPs). In this scenario, this work aims at investigating the plastic-degrading potential of microorganisms thriving in WWTP sludge, i.e. a microplastic-rich environment. The latter was used as the microbial inoculum for five enrichment batch cultures in a medium containing post-consumer polyethylene terephthalate (PET) or polylactic acid (PLA), at 37°C and 50°C. Nutrients content in the media was gradually reduced until PET or PLA were the only available carbon source and subsequently, the biofilm-forming and planktonic communities were isolated (M.Y. Kim et al., 2017, J. Microbiol. Biotechnol., 27(2), 342-349). The consortia were functionally screened for enzymatic activity associated with polyester hydrolysis (e.g., esterase, cutinase, lipase), using triglyceride emulsifiable substrates, i.e., tributyrin and coconut oil. We identified 148 isolates active on tributyrin (esterase activity), while only 25 isolates also showed lipase activity on coconut oil-containing agar plates (Charnock, 2021, J Microbiol Meth, 185, 106222). Esterase activity was also confirmed in cell-free supernatants by measuring the hydrolysis of chromogenic substrates (e.g., p-Nitrophenyl Butyrate) after a preliminary optimization of enzyme secretion. The metagenomes of the five consortia as well as that of the initial inoculum were

sequenced and will be analyzed with the aim of discovering enzyme candidates for polyester degradation.

Lars-Hendrik Köppl Poster #32
University of Freiburg, Germany

Current research topics: Interchange with other researchers from university and industry

SAHH or SIHH: Uncovering the Substrate Preferences of Methylation's Regulator

Lars-Hendrik Köppl[1], Désirée Popadić [1], Raspudin Saleem-Batcha[1], Jennifer N. Andexer[1]
[1]University of Freiburg, Institute of PharPmaceutical Sciences Albertstr. 25, 79104 Freiburg, Germany lars-hendrik.koeppl@pharmazie.uni-freiburg.de

S-Adenosyl-L-homocysteine hydrolase (SAHH) catalyses the reversible cleavage of S-adenosyl-L-homocysteine (SAH), a by-product and potent feedback-inhibitor of S-adenosyl-L-methionine (SAM) dependent methylation reactions (Figure 1) [1,2]. Their regulatory role in the methylation potential of living cells from eukaryotes, bacteria and archaea makes SAHHs an interesting target for drug development [3]. In Methanocaldococcus jannaschii, an alternative pathway for recycling of SAM metabolites was elucidated, including a 5'-deoxyadenosine deaminase (DadD) followed by a SAHH homologue with a preference for S-inosyl-L-homocysteine (SIH), the product of SAH deamination [4]. This discovery steered us to biochemically characterise various SAHH homologues from organisms of different kingdoms or phyla within the three domains of life. Our studies revealed that some homologues from eukaryotes, bacteria and archaea are catalytically active with SIH. Enzymes from Euryarchaea and evolutionary closely related thermophilic bacteria exhibit a notable substrate preference for SIH. To identify the reason for different substrate preferences within SAHHs/SIHHs, protein crystallisation and structure determination were applied together with bioinformatical methods for sequence comparison, which led to the identification of a signature sequence differing between enzymes with different substrate preferences. In addition, the first

crystal structures of archaeal SAHHs in complex with inosine or SIH offered insights into the binding mode of those untypical substrates [5].

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Markus Krause Poster #33
University of Freiburg, Germany

Current research topics: PLP-dependent alpha-oxoamine synthases

Skipping the diketone: AOS enzymatic activity generates novel C–C bond in biocatalytic cathinone synthesis

Phenylalkylamines such as ephedrine, cathinone and their derivatives are pharmaceutically active alkaloids produced by plants, for instance Ephedra sp., Pinellia ternata or Catha edulis.[1] Although commonly utilized in traditional medicine or modern medication, the biosynthetic pathway of ephedrine and cathinone remains to be elucidated. Currently, most studies on the putative biosynthetic route are based on a transaminase reaction of the aromatic diketone 1-phenylpropane-1,2-dione originated from a benzoyl derivative substrate. However, a respective gene regarding the proposed biosynthesis has not yet been identified.[2] We hypothesize a contrary biosynthetic route circumventing the transamination. The pyridoxal phosphate-dependent enzymes of the α-oxoamine synthase (AOS) enzyme family are capable of performing a decarboxylative claisen-like condensation that generates an α-oxoamine. As a member of this enzyme family the promiscuous TTHA1582 from Thermus thermophilus was used in our studies.[3] Our experiments with TTHA1582 show the conversion of a variety of aromatic thioesters with different amino acids. Focusing on cathinone, an α-oxoamine in the biocatalytic route, the formation succeeds in the conversion of benzoyl-CoA or benzoyl-SNAC with L-alanine. Hence, a putative alternative route to ephedra alkaloids in one enzymatic step is indicated.

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Dr. Cristina Ciobanu **Poster #34**
Nicolae Testemițanu State University of Medicine and Pharmacy, Moldova

Current research topics: Biopharmaceutical evaluation of drugs

Biotechnology Education for pharmacy students from the Republic of Moldava

Due to the rapid growth, the importance of biopharmaceuticals, and the techniques of biotechnologies to modern medicine and the life sciences, the field of pharmaceutical biotechnology has become an increasingly important component in the education of today's and tomorrow's pharmacists and pharmaceutical scientists. Teaching and learning about biotechnology, and the resulting skills, are known to increase students' interest in and motivation to study both biotechnology topics and science in general Thanks to advances in many different areas, including molecular biology, bioinformatics, and bioprocess engineering, we have moved from an animal-/human-derived therapeutic protein product towards in vitro- produced therapeutic proteins with fully human sequence and structure. Today, biotechnology is involved in the manufacturing process of more than 20% of all marketed drugs. More than 350 million patients worldwide benefit from healthcare biotechnology, using medicines to treat and prevent chronic diseases, including heart attack, stroke, multiple sclerosis, many cancers, cystic fibrosis, leukemia, diabetes, hepatitis, and other rare or infectious diseases. The development of applied biotechnologies in the pharmaceutical field must be based on the European model for obtaining biotechnological products (pharmaceutical bioproducts – biosimilars, cytokines, hormones, vaccines, antibodies, blood substitutes, in vitro multiplication of medicinal plants, obtaining natural compounds under controlled conditions). It requires the training of the new generation of specialists with practical knowledge and skills and requires pharmaceutical education to train new generations of specialists through learning and research. Training new specialists with biotechnological skills would be a real opportunity for international collaboration and mobility, both for teaching staff and for students, residents, and Ph.D. students involved in biotechnological research. The world practice with reference to the development of biotechnology in the field of drugs demonstrates efficiency, sustainability, and potential to reduce healthcare costs while improving patient care and complex approach in personalized medicine.

Romanos Siaperas Poster #36
National Technical University of Athens, Greece

Current research topics: Biocatalysis, Bioinformatics

Exploring the Enzymatic Machinery of a Plastic-degrading Fungus with Secretomic and Transcriptomic data

Plastics constitute the most prevalent artificial polymers in our modern societies and the properties of plastic that were sought after for decades, i.e. mainly resistance and durability, become a problem today. In recent years, public databases containing genomic data on various microbial species involved in carbon recycling have grown considerably, thereby providing a rich source for the discovery of novel enzymes. However, relying solely on sequence alignment-based algorithms can limit the discovery of enzymes with low sequence similarity to known counterparts. Therefore, incorporating proteomic and transcriptomic data is crucial for making informed decisions regarding which enzymes to study. After screening a fungal library, Fusarium oxysporum BPOP18 was chosen because it can grow using Impranil® DLN-SD as its sole carbon source. Based on assays measuring extracellular esterase activity, which is associated with plastic-degrading enzymes, we selected two time points for RNA-seq and for MS/MS analysis of the secretome. Analysis of gene and protein expression using RNA-seq and mass spectrometry will provide a more comprehensive understanding of the enzymatic machinery of Fusarium oxysporum BPOP18 and enable targeted selection of novel enzymes associated with its bioremediation potential.

Hadjira Bounabi Poster #37
Ecole Nationale Supérieure de Biotechnologie, Algeria

Current research topics: Plastic biodegradation

Exploring Algerian Ecosystems: Unraveling New Avenues for Plastic Biodegradation through Enzyme Discovery

Plastic pollution is a global environmental concern, and efforts to address this issue require the discovery of effective plastic-degrading enzymes and microorganisms. New solutions are urgently needed to address the thermolability and low activity commonly found in most enzymes capable of degrading plastics. This can be achieved by exploring nature to uncover potential enzymes or by protein engineering to enhance the performance and thermostability of the active enzymes. Research on plastic-degrading enzymes and microorganisms from Algeria is limited, therefore, this study focuses on exploring Algerian biotopes, including hot springs, soils and Sahara desert regions, to uncover new plastic-degrading enzymes using culture-dependent methods and metagenomics. These environments were chosen due to their potential for harboring thermostable enzymes suitable for plastic biodegradation. Initially, 145 bacterial isolates from different habitats were screened for their ability to degrade Bis(2-Hydroxyethyl) terephthalate (BHET) and polycaprolactone (PCL). Furthermore, genomic DNA was extracted from environmental samples and active bacterial isolates, providing data for future sequence-based identification and characterization of plastic-degrading enzymes. This research aligns with the trend of exploiting extreme ecosystems for biotechnological purposes and contributes insights into plastic biodegradation in African ecosystems. The findings will contribute to effective solutions for combating plastic pollution.

Robert Dierkes Poster #38
University of Hamburg, Germany

Current research topics: PET-Degradation, Plastic degradation, PETase activity assays, Biosensor, *Comamonas thiooxidans*

Development of a reporter strain for the detection of terephthalic acid (TPA) in environmental and industrial samples.

Robert F. Dierkes[1], Pablo Perez-Garcia[1,2], Jennifer Chow[1] and Wolfgang R. Streit[1*]
[1]Department of Microbiology and Biotechnology, Biocenter Klein Flottbek, University of Hamburg, Hamburg, Germany
[2]Christian Albrechts University Kiel, Institute for General Microbiology, Molecular Microbiology, Kiel, Germany

Polyethylene terephthalate (PET) is a prevalent synthetic polymer that is known to contaminate marine and terrestrial environments. Currently only a limited number of PET-active microorganisms and enzymes (PETases) are known [1,2]. This can in part be attributed to the lack of rapid, specific and highly sensitive detection assays for PET degradation. Here we report on the construction of a sfGFP-based reporter strain established from Comamonas thiooxidans strain S23. This strain is capable to incorporate and metabolize terephthalic acid (TPA), which is a main degradation product of PET. The respective genes for catabolism are encoded in a conserved operon ranging from tphC-tphA1 under control of TphR, an IclR-type transcriptional regulator. TPA induces transcription by interaction with TphR on a promoter region upstream of the tphC gene [3].

Here we show that C. thiooxidans S23, carrying sfGFP fused to the tphC promoter, gives a fluorescent signal at TPA concentrations in the lower micromolar range. Furthermore, a deletion mutant lacking the genomic tph cluster is capable to sense TPA in the lower nanomolar range. Using these reporter strains, it was possible to indicate the degradation activity of hydrolases on PET-substrates. The use of such sensitive TPA reporter strains could further accelerate the discovery and screening of novel PETase candidates by shortening screening durations, reducing screening costs and facilitating TPA detection in natural environments with possibly less homogenous distributions of TPA.

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Myllena Pereira Silverio Poster #39
University of Hamburg, Germany

Current research topics: Establishment of a fast and reliable assay to detect protein adsorption towards PET, Translational fusion of SusD with fluorescence labels and enzymes, Recombinant protein expression and protein characterization, Cloning of protein fragments and binding characterization with PET, Protein phylogeny

Characterization of SusD-like proteins regarding their adsorption to distinct polymers

Myllena Pereira Silverio[1], Tabea Neumann[1], Pablo Pérez-García[1,2], Jennifer Chow[1], Wolfgang Streit[1]
[1]Department of Microbiology and Biotechnology, University of Hamburg, Hamburg, Germany
[2]Molecular Microbiology, Institute for General Microbiology, Kiel University, Kiel, Germany

SusD proteins belong to the operon *sus*, largely identified in the Phylum Bacteroidota. These proteins have an important role in nutrient acquisition, being responsible for the adsorption to a range of natural polymers. Until now, SusD-like proteins were never characterized regarding their capability to bind synthetic polymers, such as PET. In this work, three SusD homologs (named SusD1, SusD38489 and SusD70111) from cow rumen and elephant faeces metagenomes were described. The signal peptide Sec/SPII, located on the N-terminal, was removed from each protein. Employing translational fusions with the fluorescence label superfolder GFP (sfGFP), 20 µM of protein was incubated with microcrystalline cellulose (MC) or chitin, which are the most common natural polymers on earth. SusD38489 and SusD1 bound MC, while SusD70111 bound better to chitin. The synthetic polymers couldn't be analyzed with this approach, once sfGFP alone presented a high binding background. However, pull-down assays gave first evidence of putative binding to polyethylene terephthalate (PET) and other synthetic polymers, such as polyamide 6 (PA6) and low-density polyethylene (LDPE). Every test was performed in triplicate.

Marno Gurschke Poster #40
University of Hamburg, Germany

Current research topics: PET-degradation, enzyme screening and optimization, MHETase/BHETase activity, enzyme promiscuity, HPLC, ferulic acid esterase, substrate characterisation

An archaeal lid-containing feruloyl-esterase degrades polyethylene terephthalate (PET)

Polyethylene terephthalate (PET) is a commodity polymer known to globally contaminate marine and terrestrial environments. Today, around 60 bacterial and fungal PET-active enzymes (PETases) are known, originating from four bacterial and two fungal phyla. In contrast, no archaeal enzyme has been identified to degrade PET. Here we report on the structural and biochemical characterization of PET46, an archaeal promiscuous feruloyl esterase exhibiting degradation activity on PET comparable to IsPETase and LCC, and higher activity on bis-, and mono-(2-hydroxyethyl) terephthalate (BHET and MHET). The enzyme, found by a sequence-based metagenome search, was derived from a non-cultivated, deep-sea Candidatus Bathyarchaeota archaeon. Biochemical characterization demonstrated that PET46 is a promiscuous, heat-adapted hydrolase. Its crystal structure was solved at a resolution of 1.71 Å. It shares the core alpha/beta-hydrolase fold with bacterial PETases, but contains a unique lid common in feruloyl esterases, which is involved in substrate binding. Thus, our study widens the currently known diversity of PET-hydrolyzing enzymes, by demonstrating PET depolymerization by a plant cell wall-degrading esterase.

Alan Wypych Poster #41
University of Hamburg, Germany

Current research topics: Polyamid degradation, Reporter strain development, Metagenomics, Plastics

Screening and Characterisation of Novel Polyamide-Degrading-Enzymes from Environmental Microbiota for Sustainable Plastic Waste Management

Polyamides (PA) are polymers consisting of repeating units linked via amide bonds. The various types of PAs, e.g. PA6(6), Kevlar©, allow a wide range of applications, which is reflected in the increasing annual world demand (MacLeod et al., 2021) . According to the established plastic-active database Pazy, currently 12 polyamidases are listed (Buchholz et al., 2021). The main investigated polyamidases are the NylA,B,C hydrolases from Paenathrobacter ureafaciens, that are active on PA oligomers (Kakudo et al., 1993; Negoro et al., 1992; Okada et al., 1983) . Until now, no microorganisms have been identified with the capability of full degradation of a high molecular weight polymer. Here we report the identification of novel, putative polyamidases from environmental metagenomes that show activity on PA6-polymer. Furthermore, the marine bacterium species NDB-UHH1 was identified, which is able to use PA-monomers as C-source.

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