Program

Time: November 10, 2014, Monday
Venue: Lecture Hall 214, QIBEBT

Chair	**Dr. LU Xuefeng,** deputy director general, QIBEBT
08:30-08:50	**Title:** Gas fermentation using autotrophic acetogenic bacteria for production of chemicals and fuels **Dr. Peter Dürre,** Director, Institute of Microbiology and Biotechnology, University of Ulm
08:50-09:10	**Title:** Distinct Roles for Carbohydrate-binding Module in Families 10 and 11 Xylanases from Caldicellulosiruptor sp. F32 **Dr. LI Fuli,** QIBEBT
09:10-09:30	**Title:** Glycerol and glucose as renewable carbon sources for the Metabolic Engineering of *Escherichia coli* towards the production of amino acids, vitamins, milk oligosaccharides, and rare fine chemicals **Dr. Georg Sprenger,** University of Stuttgart, Institute of Microbiology, Stuttgart, Germany
09:30-09:50	**Title:** Genetic engineering of cellulosome-producing Clostridium strains for lignocellulosic biomass Biorefinery **Dr. LIU Yajun,** QIBEBT
09:50-10:10	**Title:** Towards a “bioeconomy”-Programs in the EU, Germany and Baden-Wurttemberg **Dr. Rolf D Schmid,** emeritus Stuttgart University, CLIB2021 Advisory Board Member, bioeconomy strategy of Baden-Wurttemberg
10:10-10:30	**Title:** Attached biofilm cultivation practice for *Spirulina platensis* **Dr. LIU Tianzhong,** QIBEBT
10:30-10:50	**Coffee break**
Chair	**Dr. Rolf D Schmid,** emeritus Stuttgart University, CLIB2021 Advisory Board Member, bioeconomy strategy of Baden-Wurttemberg
10:50-11:10	**Title:** Microbial production of platform chemicals from renewable sources **Dr. Ochsenreither Katrin,** Karlsruhe Institute of Technology, Institute of Process Engineering in Life Science
11:10-11:30	**Title:** Efficient Biofuel Cell Based on Novel Nanostructures and Microbial Surface Displaying Enzyme **Dr. LIU Aihua,** QIBEBT
11:30-11:50	**Title:** Microfluidic platforms for high throughput microbial screening **Dr. MA Bo,** QIBEBT
11:50-12:10	**Title:** Insilico Biotechnology – In silico solutions for industrial biotechnology **Dr. Klaus Mauch,** Insilico’s co-founder , CEO
12:10-12:30	**Title:** In silico Parts Discovery from Microbial Communities **Dr. NING Kang,** QIBEBT
12:30-13:30	**Lunch**
13:30-15:20	**Discussion**

Title: Gas fermentation using autotrophic acetogenic bacteria for production of chemicals and fuels

Dr. Peter Dürre

Director, Institute of Microbiology and Biotechnology, University of Ulm

Abstract

Autotrophic acetogenic bacteria employ the so-called Wood-Ljungdahl pathway for growth, forming naturally acetate, ethanol, and/or 2,3-butanediol from gaseous substrates such as CO2 + H2 or syngas (mostly a mixture of CO + H2). To date, different acetogens are used in industrial applications in pilot and demonstration plants aiming at ethanol formation from different syngas sources. A major challenge is to reengineer these bacteria metabolically for formation of other interesting chemicals, allowing fermentation with an abundant, cheap carbon source and, in parallel, even consumption of greenhouse gases.

Clostridium ljungdahlii is such an acetogen, able to ferment either organic compounds or CO2 + H2 and syngas (CO + H2). The genome of *C. ljungdahlii* comprises 4,630,065 base pairs. Experimental data and *in silico* comparisons revealed differences in energy metabolism. Unlike *Moorella thermoacetica*, no cytochromes and quinones are involved in energy generation, but instead an H+-dependent Rnf system is present, analogous to *Acetobacterium woodii* with a Na+-dependent Rnf system. Electroporation of *C. ljungdahlii* with plasmids bearing heterologous genes for butanol production was successful and formation of the biofuel could be demonstrated. Thus, *C. ljungdahlii* can be used as a novel microbial production platform based on syngas.

As the organism does not grow well on CO2 + H2 mixtures, *Clostridium aceticum* was chosen for this type of gaseous substrate. Expression of both, heterologous butanol- and acetone-forming enzymes could be demonstrated. Genome sequencing of this species is currently being performed. *C. aceticum* can also use syngas as a carbon source.

Finally, *A. woodii* was improved for acetate formation from CO2 + H2 by introducing and overexpressing clostridial genes encoding Wood-Ljungdahl pathway enzymes.

Title: Distinct roles for carbohydrate-binding module in families 10 and 11 Xylanases from *Caldicellulosiruptor* sp. F32

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Abstract

Xylanases are crucial for lignocellulosic biomass deconstruction and generally contain non-catalytic carbohydrate-binding modules (CBMs) accessing recalcitrant polymers. Understanding how CBMs affect protein catalytic efficiency and thermostability would provide new insight into protein intra-molecular interactions. Truncated mutant of glycoside hydrolase (GH) family 11 XynA lacking CBM exhibited a considerable improvement in specific activity and thermal stability compared with the wild type. However, XynB, a GH10 xylanase with 2 CBMs, showed higher enzyme activity and thermostability than its truncated mutant without CBM. Through homology modeling and cross-linking experiments analysis, we demonstrated that for GH10 XynB, the resistance against thermoinactivation was generally affected by both domain properties and inter-domain interactions, whereas for GH11 xylanase XynA, no inter-domain interactions were observed. Inter-domain interaction can accelerate thermostability and catalytic efficiency. We speculated optimized intra-molecular interaction was determined by catalytic domain and CBM through fine tuning rigidity and flexibility of linker, which might provide microbes a powerful evolution strategy to assemble catalyst functioning under various ecological conditions.

Title: Glycerol and glucose as renewable carbon sources for the metabolic engineering of *Escherichia coli* towards the production of amino acids, vitamins, milk oligosaccharides, and rare fine chemicals

Georg Sprenger
University of Stuttgart, Institute of Microbiology, Stuttgart, Germany

Abstract

Escherichia coli K-12 is a model organism in microbiology and genetics. In biotechnology, *E. coli* is also used for the production of many amino acids, vitamins and fine chemicals. We have developed *E. coli* strains which can be used for the production of aromatic amino acids (L-phenylalanine [1], L-tryptophan), of fine chemicals as building blocks for pharmaceuticals (aminocyclitols, transdiols) [2], of bioactive compounds (violacein) [3], carotenoids (lycopene, astaxanthin) [4,5], vitamin E (-tocotrienol) [6], or human milk oligosaccharides such as 2-fucosyllactose [7] or lacto-*N*-tetraose [8]. We will present examples of our recent work on *E. coli* strain improvement with emphasis on stable chromosomal modifications [9].

Whereas glucose is the common carbon source for most productions with *E. coli*, glycerol and crude glycerol (a waste product of biodiesel production) has attained much interest in recent years. Crude glycerol could be used successfully for the production of L-phenylalanine [10-12]. However, several enzymatic steps in glycolysis, gluconeogenesis and in the pentose phosphate metabolism had to be improved therefore [13]. We will present and discuss recent results of this.

[1] Sprenger G.A. 2007. *Appl. Microbiol. Biotechnol*. 75: 739-749.
[2] Bongaerts J. 2011. *Angew. Chemie Intl. Ed.* 50: 7781-7786.
[3] Rodrigues AL.et al. 2013. *Metabol. Engin*. 20: 29-41.
[4] Albermann C. 2011. *Biotechnol. Lett.* 33: 313-319.
[5] Lemuth K. et al. 2011. *Microb. Cell Fact.* 10: 29.
[6] Albermann C. et al. 2008. *ChemBioChem*, 9: 2524-2533.
[7] Baumgärtner F et al. 2013. *Microb. Cell Fact.* 12:40.
[8] Baumgärtner F. et al. 2014. *ChemBioChem*, 15: 1896-1900.
[9] Albermann C. et al. 2010. *Biotechnol J.* 5: 32-38.
[10] Weiner M. et al. 2014. *Biochem. Engin. J.* 83: 62-69.
[11] Weiner M. et al. *Biotechnol. Bioengin.* 111: 1406-1416.
[12] Weiner M. et al. 2014. *Biotechnol. Bioengin*. 111: in press.
[13] Gottlieb K. et al. 2014. *Microb. Cell Fact.* 13: 96.

Title: Genetic engineering of cellulosome-producing *Clostridium* strains for lignocellulosic biomass biorefinery

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Abstract

Clostridium thermocellum and *Clostridium cellulolyticum* are typical cellulosome-producing *Clostridium* strains that can produce ethanol from cellulosic substrates, and have been considered as the most promising candidates for bioconversion of lignocellulose and production of bioethanol via consolidated bioprocessing strategy. Nevertheless, metabolic engineering of the wild-type strains has to be performed to meet the requirement of industrialization, which is delayed by the lack of genetic tools for *Clostridium* strains. Besides, cellulosome is a macromolecular complex consisting of multiple cellulose degrading enzymes organized and attached to the cell surface by non-catalytic scaffoldins, while without efficient methods for genetic manipulation of cellulosome-producing *Clostridium*, how different scaffoldins and their functional modules contribute to cellulose hydrolysis has remained unclear.

To achieve the genetic engineering of cellulosome-producing *Clostridium* efficiently and precisely, we firstly developed a series of genetic tools using mesophilic *C. cellulolyticum* H10 and thermophilic *C. thermocellum* DSM1313 as model strains, with which the metabolic engineering of these strains was performed and significantly enhanced the ethanol production.

To deeply understand the structure-activity of cellulosome, we analyzed the contribution of different scaffoldin and modules of cellulosome in cellulose degradation by constructing *C. thermocellum* mutants with truncated primary scaffoldin CipA or disrupted secondary scaffoldins. Our results demonstrated the major role of cellulosome-substrate synergy and a relatively small contribution of cellulosome-cell synergy. Surprisingly, mutants lacking cell-associated polycellulosomes adheres to cellulose almost as strongly as wild-type cells, revealing an alternate, previously unknown cellulose-binding mechanism.

Title: Towards a "bioeconomy"-programs in the EU, Germany and Baden-Wurttemberg

Rolf D Schmid

Emeritus Stuttgart University, CLIB2021 Advisory Board Member, bioeconomy strategy of Baden-Wurttemberg

Abstract

In an industrial society such as Western Europe innovation comes not only from new products, but also from novel manufacturing routes which are independent from fossile energy sources and raw materials and which are environmentally benign. The European Union, in its Horizon 2020 program, has set aside a budget of several billion € to support such programs, partially through industrial activities such as the Bio-based Industries Consortium (BIC).

Germany, in 2010, started on a National Research Strategy BioEconomy 2030 which has many elements, e. g., a Roadmap to Biorefineries which has already led to operating facilities.

Baden-Wurttemberg, the most advanced state in the Southwest of the German Federation, has started her own specific program looking at bioeconomy in value-added chains and as an integrated system. In this program, economics, social sciences and bioethics will be integrated, and a system-oriented approach is being done which integrates agricultural and forestrial raw materials as well as biogenic waste and food.

A brief survey on these programs and activities will be provided.

Title: Attached biofilm cultivation practice for *Spirulina platensis*

ZHANG Lanlan, CHEN Lin, CHEN Yu, WANG Junfeng and LIU Tianzhong
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Abstract

Microalgae *Spirulina platensis* was cultivated with the "attached biofilm cultivation" technique in both laboratory and outdoor bench bioreactor. Through the laboratory investigations, an appropriate attached biofilm cultivation condition was suggested as 7.8~11g m^{-2} for initial inoculum density, lower than 200 μmol m^{-2} s^{-1} irradiation, 0.5% of CO_2 concentration and 0.0056m/s superficial aeration rate with $NaHCO_3$-free Zarrouk medium. An outdoor bench reactor based on 10 times light dilution was constructed and 10 days cultivation with harvesting every day was carried out. High footprint areal biomass productivity of around 60 g m^{-2} d^{-1}, or photosynthetic efficiency of 8.9~14.5% was achieved. The results has shown the promising potential of attached biofilm cultivation technology in solving the cultivation efficiency and harvesting difficulty for microalgae.

Title: Microbial production of platform chemicals from renewable sources

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Abstract

Crude Oil and natural gas are the main sources for energy supply, fine chemicals and raw materials for the chemical industry as 95 % of the worldwide primary building blocks for organic chemicals originate from them. In times of rapid oil depletion and climatic change it is urgently needed to find sustainable and eco-friendly alternatives for both energy and chemical demands.

In our group "Technical Biology" we focus on the microbial production of several platform chemicals from renewable sources: single cell oils (SCOs), organic acids and alcohols.

SCOs are lipids produced by oleaginous microorganisms. Depending on the fatty acid profile of the SCO these lipids can be regarded as good alternatives to replace conventional oil sources for application in energy sector, food, pharmaceutical and cosmetic industry. Oleaginous microorganisms, including bacteria, yeasts, fungi and microalgae, are able to produce lipids as carbon storage with yields between 20% and 80% lipid per dry biomass. A platform process for SCO production was established with the model organism *Cryptococcus curvatus* and was applied to the newly screened oleaginous yeasts *Cryptococcus podzolicus* and *Trichosporon porosum* which are able to produce SCO on hemicellulosic material with gluconic acid as a by-product.

Dicarboxylic acids can serve as polymerization starter units due to their bifunctionality and can be easily converted to other important fine chemicals. The three 1,4-dicarboxylic acids succinic, malic and fumaric acid are not only produced by every living organism as part of the TCA-cycle but have been identified as one of the top twelve value added chemicals from biomass by the US Department of Energy. The filamentous fungus *Aspergillus oryzae* can produce large quantities of fumaric and malic acid and secrete them into the culture broth when cultured under stress conditions. To prevent the "food or fuel"-discussion which would be a problem when using glucose as carbon source the renewable carbon source xylose and the waste-substrate glycerol were successfully applied for the production of malic acid by *A. oryzae* DSM 1863.

A good strategy for the complete usage of lignocellulosic biomass is pyrolysis and gasification of the biomass in the "BioLiq" plant and the application of the resulting syngas in an anaerobic fermentation process. In our group *Clostridium ljungdahlii* and *Acetobacterium woodii* are used to convert H2, CO and CO2 to acetate, ethanol and butyrate.

Title: Efficient biofuel cell based on novel nanostructures and microbial surface displaying enzyme

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Abstract

The power output and stability of biofuel cells (BFCs) is greatly dependent on the properties of both the biocathode and bioanode. Microbial surface display is a biotechnology that protein, peptides or antibody could be expressed on the surface of microorganisms, which could find potential applications in biocatalysis, environmental governance and bioanalysis. In this talk, efficient biofuel cells based on carbon nanotubes and highly uniform three-dimensional (3D) macroporous gold (MP-Au) film as well as microbial surface displaying enzyme will be highlighted. We found that the laccase/MP-Au biocathode exihibited an onset potential of 0.62 V versus SCE (or 0.86V vs. NHE) toward O_2 reduction and a high catalytic current of 0.61 $mAcm^{-2}$. On the other hand, mutated glucose dehydrogenase (GDH) surface displayed bacteria (GDH-bacteria) were used to improve the stability of the glucose oxidation at the bioanode. The as-assembled membraneless glucose/O_2 fuel cell showed a high power output of 55.8 μW cm^{-2} and open circuit potential of 0.80 V, contributing to the improved electrocatalysis toward O_2 reduction at the laccase/MP-Au biocathode. Moreover, the BFC retained 84% of its maximal power density even after continuous operating for 55 h because of the high stability of the bacterialsurface displayed GDH mutant toward glucose oxidation. Our findings would open a new avenue for using degradation product of agricultural wastes as a direct energy source.

Title: Microfluidic platforms for high throughput microbial screening

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Abstract

Microfluidics and Lab on a chip technologies hold significant advantages on high or ultra-high throughput screening of industry enzyme and microbial at single cell level because of its low reagent consume, low cost and high throughput. Here, I will present our progress on the development of microfluidics platforms for non-invasive screening based on Raman activated cell sorting, which could be used for screening of living microbial cells with high yield chemical production. Except for the phenotype identification, to clarify the genotype of an individual cell with some specific phenotype, droplet microfluidics based single cell genomics including single cell sequencing and digital PCR technologies will be presented in this talk.

Title: Insilico Biotechnology – In silico solutions for industrial biotechnology

Klaus Mauch
Insilico's co-founder, CEO

Abstract

Quantification and prediction of cellular phenotypes via mechanistic computer models become increasingly important for knowledge-based development of biotechnological processes. Insilico Biotechnology has developed an integrated technology platform which supports industrial biotechnology in streamlining research and development by providing expertise in modelling and simulation of biochemical networks. The in silico platform comprises genome-based models and software tools for model simulation and analysis in an enterprise setting. Application areas of the technology include (i) reconstruction of genome-based simulation models, (ii) de novo design of metabolic pathways, (iii) identification of bottle-necks, and (iv) prediction of multiple gene targets for increasing product yield. For this purpose, the technology platform can take advantage of built-in stationary simulation algorithms such as flux-balance analysis as well as of dynamic methods that are based on mechanistic kinetics. Application examples will be shown to illustrate how Insilico's genome-based models and simulation technology can be used to improve decision-making, to increase process performance, and to reduce development risks and costs.

Title: In silico parts discovery from microbial communities

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Abstract

With the fast accumulation of microbial community samples and related metagenomic sequencing data, *in silico* discovery of functional parts from microbial communities has been made possible. However, this computer-aided approach has been limited by current "big-data" challenge. Therefore data integration and analysis system is urgently needed for parts mining from in-depth analysis of large scale metagenomic samples (also referred to as “microbial communities”) of interest. To facilitate comprehensive mining on large amount of diverse metagenomic samples, we have designed a Meta-Mesh system for a variety of functional part and module discoveries. Experiment results have shown that Meta-Mesh would serve as an efficient data analysis platform for *in silico* discovery of clusters, biomarkers and other functional parts from a large pool of microbial samples. Such *in silico* identification of functional parts would provide candidates that could help to boost the introduction of novel functions into chassis organisms for synthetic biology.