UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

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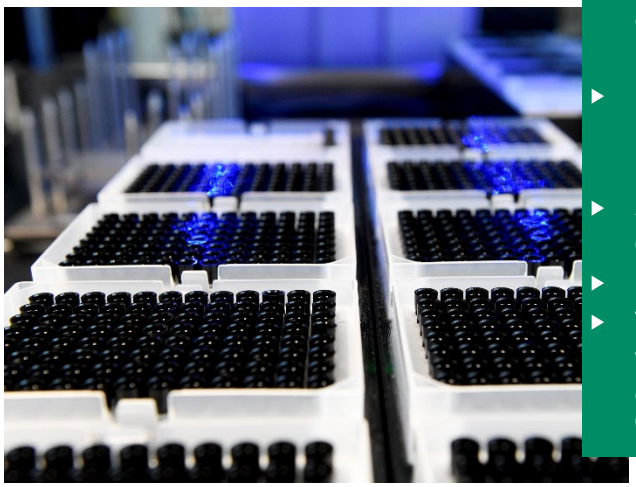

- Imine reductases & the “move” of this enzyme class from basic research to applications for pharmaceutical synthesis
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Imine reductases & the “move” of this enzyme class from basic research to applications for pharmaceutical synthesis

In the biocatalysis community, many international research groups focused in recent years successfully on evaluating the potential of imine reductases as a relatively new biocatalyst class in organic synthesis, which are suitable for the enantioselective preparation of secondary amines. Such secondary amines, which can be acyclic as well as cyclic products, are found as subunits in numerous pharmaceuticals. However, today´s production process is typically still based on “classic chemistry”. On the other hand, imine reductases open up a perspective for an alternative, highly efficient and sustainable access to such target molecules on industrial scale.

Within the field of such cyclic secondary amine molecules, CeBiTec researchers of the Chair of Industrial Organic Chemistry and Biotechnology investigated the imine reductase-catalyzed synthesis of five-membered cyclic amines with an aromatic ring as a substituent due to the pharmaceutical importance of this type of compound class (Figure 1).

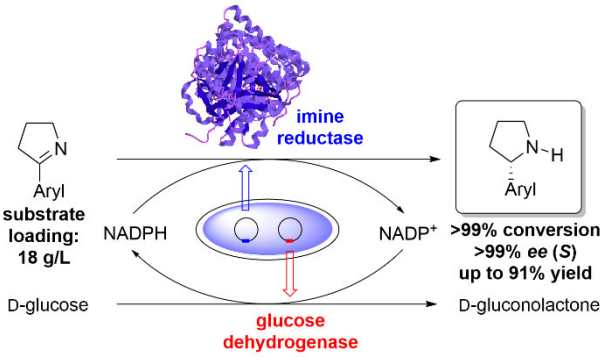

Figure 1: Scheme showing the biocatalytic concept.

Representative examples of such so-called chiral pyrrolidines are the oncology-drug Larotrectinib (Bayer) and the potential CDK8 inhibitor

MSC2530818. In a joint collaboration work of the doctoral course student Laura Bernhard and the bachelor student Jill McLachlan, an enantioselective access to the corresponding pyrrolidines was achieved by reduction of the C=N double bond in pyrrolines in the presence of a recombinant imine reductase from *Cupriavidus* sp. HPC(L) overexpressed in *Escherichia coli*. As reducing agent, D-glucose was used in combination with a glucose dehydrogenase for *in situ*-cofactor recycling. In the presence of *E. coli*-whole cell catalysts, the heterocyclic amine products were obtained with >99% conversion, an enantioselectivity of >99% ee, and yields of up to 91%. The process can be carried out at a substrate loading of 18 g/L, which already represents an attractive range also for applications on larger scale.

This research work of Laura Bernhard and Jill McLachlan has now been published by the ACS-journal "*Organic Process Research & Development*" (in an ASAP-online format), and is planned to be included in a "Biocatalysis"-special issue of this journal.

Reference

Laura M. Bernhard, Jill McLachlan, and Harald Gröger, "Process Development of Enantioselective Imine Reductase-Catalyzed Syntheses of Pharmaceutically Relevant Pyrrolidines", *Organic Process Research & Development* 2022, Article ASAP; DOI: 10.1021/acs.oprd.1c00471 (online publication date: February 28, 2022).

(H. Gröger)

11th International CeBiTec Research Conference Bielefeld on intensification of biocatalytic processes

From March 28-30, 2022, the 11th international CeBiTec Conference being entitled "Intensifying Biocatalytic Processes – From Enzyme and Material Design to Industrial Applications" (IBPRO2022) took place as a "hybrid event" at the ZiF building. This conference was jointly organized by the EU-Project (H2020-MSCA-ITN) "INTERfaces" (in which the CeBiTec is also involved with a research project) with Prof. Dr. Selin Kara (Aarhus University) as a coordinator and the research group "Industrial Organic Chemistry and Biotechnology" as a member of the CeBiTec. We were happy to welcome about 60 participants in presence and further ca. 45 participants who joined the conference in the online mode.

The program of IBPRO2022 consisted of four sessions in the areas of "Enzyme discovery and design for industrial applications", "Heterogenization of biocatalysts with tailor-made materials", "Intensification of heterogenized bioprocesses: Multicatalytic cascades and flow biocatalysis" and "Out-of-the-box methods: Future trends in biocatalysis". The presented contributions comprised lectures from 8 invited speakers coming from both academia and industry as well as in total 14 oral presentations. Furthermore, the lectures were complemented by a poster pitch session, online-poster sessions, a lecture by Dr. Ruben Ragg (editor-in-chief of ChemBioChem) about changes in the publication

landscape and two “round table”-discussions addressing career opportunities in biocatalysis as well as an “outlook” for the future development of biocatalysis. All these contributions gave exciting insights into various areas of biocatalysis, material sciences and bioprocess development, respectively, and also clearly revealed perspectives for novel processes and products through combination of these research areas.

Figure 2: Group photo with participants of IBPRO2022 in front of the ZiF building

In the following, a brief summary about the invited lectures is given: Prof. Rebecca Buller (Zurich University of Applied Sciences) illustrated the power of protein engineering by means of the development of engineered Fe(II)/α-ketoglutarate-dependent enzymes. Dr. Erika Tassano (Novartis) took us on a “journey” on the unexpected finding of a new and unprecedented biosynthetic oxidation mechanism within an enzyme screening project. Exemplified for the transaminase-catalyzed synthesis of chiral amines, Dr. Jan von Langermann (University of Rostock) demonstrated how tailor-made crystallization techniques can lead to efficient in situ-product removal, thus shifting equilibria towards the desired product side. The scope of immobilization in industrial biocatalysis was shown by Dr. Alessandra Basso (Purolite) by sharing with us numerous examples of applications on technical scale. The great potential of integrating flow chemistry into biotechnology was described Prof. Francesca Paradisi (University of Bern) exemplified the utilization of flow chemistry for multistep enzymatic cascades. Dr. Alba Diaz-Rodriguez (GlaxoSmithKline) gave an insight into the role of biocatalysis in the pharmaceutical industry. The importance of biocatalysis for fine chemicals was shown by Dr. Martin Schürmann (InnoSyn) for a range of examples of cofactor-dependent biocatalysts and oxidation reactions. How rational design of novel biocatalysts can benefit from mechanistic understanding including reactive intermediates was presented by Prof. Bernhard Hauer (University of Stuttgart) exemplified for the engineering of cyclases as promising enzymes for industrial biocatalysis.

Besides enjoying fascinating science, we also enjoyed intensive networking, to which also the evening events, in detail the welcome party on the first conference day and the conference dinner on the second day of IBPRO2022, contributed. Thus, we are grateful for all participants, who joined IBPRO2022 online and in presence. And, we are all looking forward to the further progress in this interdisciplinary field of merging biocatalysis with materials chemistry.

(H. Gröger, on behalf of the organization committee of IBPRO2022)

A bacterial platform for production of aromatic aldehydes such as vanillin

The long-term collaboration partner of the Wendisch lab, Prof. Jin-Ho Lee from Kyungsung University, Busan, South Korea, has developed a *Corynebacterium glutamicum* platform strain that allowed redirecting the carbon flux from

aromatic amino acid biosynthesis and aromatic compound catabolism towards the fermentative production of vanillin, 4-hydroxybenzaldehyde and protocatechuic aldehyde. As we immediately associate with vanillin, these aromatic aldehydes are fragrance and flavor molecules in high demand by the food and cosmetics industries.

The metabolic engineering approach efficiently yielded the aldehydes from the precursor acids as result of introducing carboxylic acid reductase from *Nocardia iowensis*.

The methylation step to vanillin was realized by introduction of catechol *O*-methyltransferase from *Rattus norvegicus* and addition of L-methionine. However, the three aldehydes accumulated only transiently in the three engineered strains before quickly being reduced to the respective alcohols. The Lee lab used a bioinformatics approach to identify putative aromatic aldehyde reductases encoded in the *C. glutamicum* genome. Next, a systematic gene deletion project was performed experimentally. The enzyme encoded by NCgl0324 turned out to be the major hit (Figure 3).

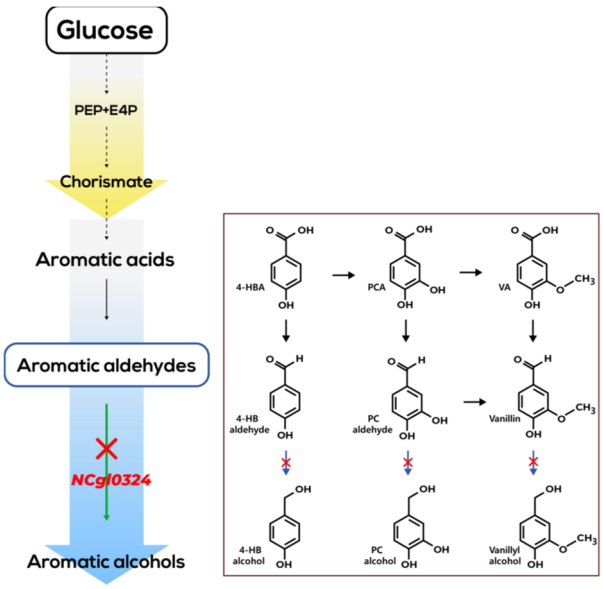

Figure 3: Scheme showing the metabolic engineering strategy and the aromatic aldehydes obtainable by its application.

Upon its deletion, production of 4-hydroxybenzaldehyde was increased by 188%. As shown by enzyme assays, the NCgl0324 protein also accepted vanillin and protocatechuic aldehyde as substrates for their NADPH-dependent reduction. In the absence of NCgl0324, production of vanillin and protocatechuic aldehyde increased to titers of about 0.3 g/L and 1.2 g/L, respectively.

The labs have exchanged MSc and PhD students for short-term research visits, including coauthor Lenny Ferrer who joined the Wendisch lab after having finished her MSc thesis project in the Lee lab. Moreover, Prof. Lee joined the Wendisch lab as visiting professor from 2016 to 2017.

Reference

Kim H-S, Choi J-A, Kim B-Y, Ferrer L, Choi J-M, Wendisch VF & Lee J-H (2022). Frontiers in Bioengineering and Biotechnology 10: 880277. DOI: 10.3389/fbioe.2022.880277

(V. F. Wendisch)

Pioneers from NRW – Home of Biotech

CeBiTec member Dr. Nadja Alina Henke participated in the new interview series “Pioneers from NRW – Home of Biotech” produced by BIO.NRW.

Figure 4: Pioneers from NRW interview series

The interview took place in November 2021 in the CeBiTec. In the interview Nadja Henke talks about the motivation and application of biotech

research. Moreover, the current challenges and chances of biotech transfer projects in NRW are discussed in the context of regional and national potential and limitations. About once every two month, the series will present different inspiring and innovative pioneers from North-Rhine Westfalia.

The series will kick off on April 26th with the interview with Dr. Henke entitled "The reddest salmon comes from Bielefeld". Her research in the CeBTec Research Area 2 is supported by the BlueBio CoFund project SIDESTREAM and Dr. Henke is leading the VIP+-project KaroTec.

(N. A. Henke)

The teutolab-biotechnology student laboratory wins the LeLa Prize - 1st place in the Experiment of the Year category with the online course "Evolution of Coronaviruses".

Forced by the Covid 19 pandemic, the teutolab-biotechnologie student laboratory switched from face-to-face learning to e-learning and developed a large online learning platform with online self-learning offers as well as various online experimentation courses transmitted via video conference. The teutolab-biotechnology has now received a special award for the course "Evolution of Coronaviruses". The association of German student laboratories LernortLabor (short: LeLa) honors outstanding achievements of student laboratories in various categories once a year. At this year's LeLa conference, teutolab-biotechnologie was awarded first place in the Experiment of the Year category for its "Evolution of Coronaviruses" course. This course is aimed at biology students in the upper secondary school level and links the two subject areas of genetics and evolution, embedding them in a current and relevant context (current pandemic). Since October 2020, this course has been conducted 38 times with a total of about 600 young people (status April 2022). It is accompanied via videoconference from the lab (duration 4 h) and combines interactive e-learning modules, lectured content, independent work with bioinformatics software, and group and plenary discussions.

The students work independently with the software MEGA (Molecular Evolutionary Genetics Analysis) and perform genetic analyses (analysis of gene mutations), phylogenetic analysis (creation and analysis of phylogenetic trees) and design a suitable gene probe for a diagnostic PCR for a diagnostic PCR infection detection.

Figure 5: The teutolab-biotechnology team

The course was evaluated by teachers and students as being very useful (qualitative/ quantitative online evaluation). Especially the independent work with the interactive content and the MEGA software as well as the current context was very appealing to the young people. This course shows how closely current scientific research can be linked to school education. In particular, the jury appreciated that by taking

the course, students learn to participate in the social and political debates around the pandemic issue in a scientifically sound way. The course is currently being revised so that, in addition to the online course, it can also be offered as a face-to-face course at teutolab-biotechnologie. The course development was financially supported by the „Europäischer Fonds für Regionale Entwicklung (EFRE)“.

(M. Panhorst)

Impressum

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Figures:
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