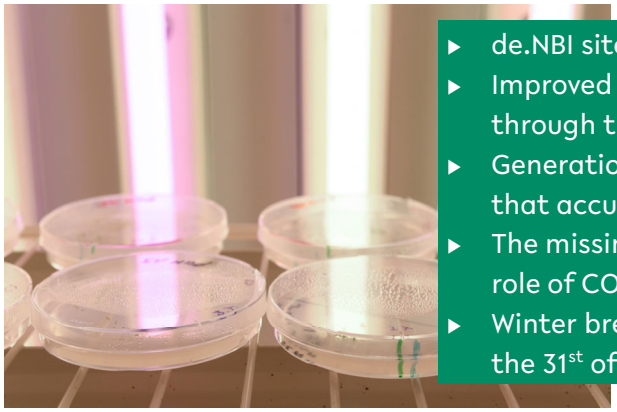
CeBiTec – Quarterly

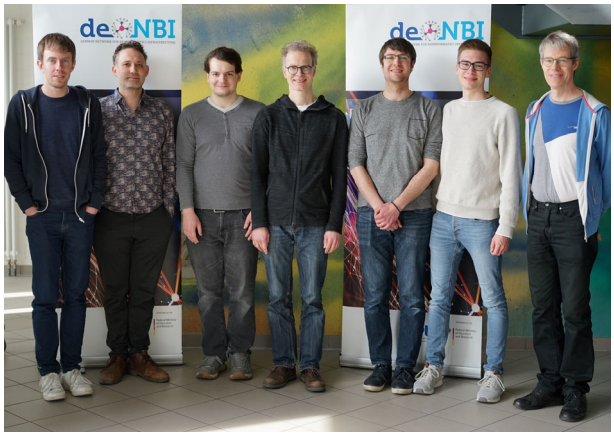
Spring 2024



- de.NBI site Bielefeld continues its work
- Improved rapeseed protein for human consumption through the reduction of bitter-tasting compounds
- Generation of a novel *Arabidopsis thaliana* triple mutant that accumulates eriodictyol derivatives
- The missing link of coenzyme Q biosynthesis identified: role of COQ4 in humans
- Winter brewing competition of the Technical Faculty on the 31st of January

de.NBI site Bielefeld continues its work

After the continuation of the de.NBI project has been established at the Forschungszentrum Jülich during the last two years, we are happy to announce that also the de.NBI site at Bielefeld University and the CeBiTec has been revived at the end of 2023 under the new acronym “MicroGenUniBi” (de.NBI-Resource Center for Microbial Genome Research in Biotechnology and Medicine at Bielefeld University). Daniel Göbel, Rainer Orth, Andreas Rempel and Tizian Schulz are now forming the new team to develop, maintain and offer the services provided at CeBiTec for the de.NBI network and ELIXIR Germany. The project has even been expanded by the working group of Robert Heyer who will enrich the service catalogue with software solutions for multiomics data analyses. For de.NBI-related work Daniel Kautzner will join Robert’s group as a new employee.



The MicroGenUniBi team; © T. Schulz

In close collaboration with the groups of Alexander Sczyrba at FZ Jülich (IBG-5) and Alexander Goesmann at JLU Gießen, MicroGenUniBi will ensure further operation of the de.NBI cloud site at Bielefeld University. This involves the installation and maintenance of hardware components, the configuration of software and the (re)certification of the cloud infrastructure. Furthermore, MicroGenUniBi provides services in

microbial bioinformatics and offers training courses introducing the usage of these services regularly. Currently, provided services cover areas of pangenomics, metagenomics and multiomics analyses. In particular, the area of pangenomics is represented by the services “PLAST” for pangenome alignment, “Corer” for the prediction of a pangenome’s core genome and “SANS” for phylogenetic analyses. The programs “QuPE”, “MeltDB” and “Fusion” cover the area of multiomics analyses. In addition, MicroGenUniBi provides various software solutions, e.g. for the computation of alignments, the comparison of genomes and RNA structure prediction which are collectively available from the platform “BiBiServ”. Moreover, the web-based software “BIIGLE” for collaborative image and video annotation is offered, specifically for biodiversity research.

For the area of metaproteomics, MicroGenUniBi provides the “MetaProteomeAnalyzer” as an intuitive open-source tool for the analysis and interpretation of metaproteome data. It contains several search engines as well as functionnality to group redundant, homologous proteins and the taxonomic and functional evaluation of microbiomes. An extension of this tool, the “MPA_Pathway_Tool” enables a flexible creation of metabolic networks and the linkage of such to proteome data. Perspectively, it is also planned to integrate the software “STAMPS” of the ISAS institute in Dortmund into this tool to ensure an ongoing availability of STAMPS.

(T. Schulz, J. Stoye & R. Heyer)

Innovation in rapeseed breeding: improved rapeseed protein for human consumption through the reduction of bitter-tasting compounds

Rapeseed protein is currently utilized at most as an additive in animal feed due to its bitter taste. This off-taste fundamentally restricts its use as a food product and has thus hindered commercialization so far. To enable broad-scale usage of rapeseed protein for human consumption, the reduction of bitter compounds present in rapeseed is therefore of high importance. Kaempferol derivatives are known as bitter-tasting compounds in rapeseed protein extracts. Thus, by reducing or eliminating kaempferols in rapeseed seeds, the taste and quality of rapeseed protein can be significantly improved.

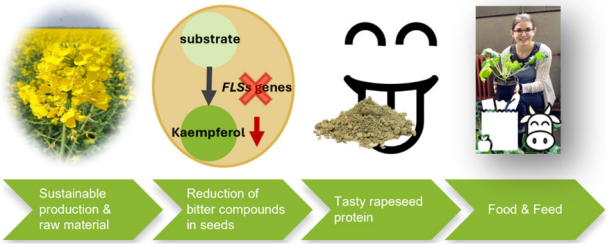


Figure 1 | Identification of rapeseed lines and seeds with reduced bitter-tasting Kaempferol content. © H. Schilbert

Through the identification of rapeseed TILLING lines carrying mutations in the two key genes, *Flavonol Synthase 1-1* and *Flavonol Synthase 1-2*, the content of bitter-tasting kaempferols was reduced by 99%. This invention has been patented by the BitRaps team consisting of the Bielefeld/CeBiTec scientists Dr. Hanna M. Schilbert, Dr. Daniela Holtgräwe, Prof. Dr. Bernd Weisshaar, and the plant breeding researchers Dr. Frank P. Wolter and Dr. Amine Abbadi. In Germany alone, on a cultivation area of ~1 million hectares, 770,000 tons of rapeseed protein could be obtained,

thereby replacing over 50% of the protein currently consumed through animal products. The concurrent use of rapeseed as an oil and protein plant is also environmentally sustainable, as existing cultivation areas are utilized, and imports of alternative plant protein sources can be reduced.

(H. Schilbert, D. Holtgräwe & B. Weisshaar)

Generation of a novel *Arabidopsis thaliana* triple mutant that accumulates eriodictyol derivatives, providing opportunities for food supplement development.

Flavonoids, a class of specialized plant metabolites, have many beneficial properties for both plants and humans. For example, they can serve as dietary supplements. Their biosynthesis involves several modification steps, which contribute to the remarkable diversity of flavonoids observed in plant species. Central to this modification process are the 2-oxoglutarate-dependent dioxygenases (2-ODDs) flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), and anthocyanidin synthase (ANS). These enzymes are highly similar and have been shown to catalyse, at least in part, each other's reactions. However, little is known about such *in planta* bypass reactions and the flavonoid composition of plants lacking all three central flavonoid 2-ODDs.

Members of the Weisshaar group addressed this issue by generating a *f3h/fls1/ans* triple mutant. This triple mutant and corresponding double mutants were investigated in detail with respect to the flavonoids accumulated in the members of this new mutant collection.

Genomic, transcriptomic and metabolomic analyses were used to characterise the impact of the mutations on flavonoid accumulation and bypass reactions. The findings reveal the *in planta* multifunctionality of F3H, FLS1 and ANS, demonstrating their ability to catalyze overlapping reactions in the absence of their counterparts. Remarkably, the *f3h/fls1/ans* triple mutant exhibited a bypass pathway leading to the accumulation of eriodictyol derivatives, driven by flavonoid 3'-hydroxylase (F3'H) activity (Figure 2).

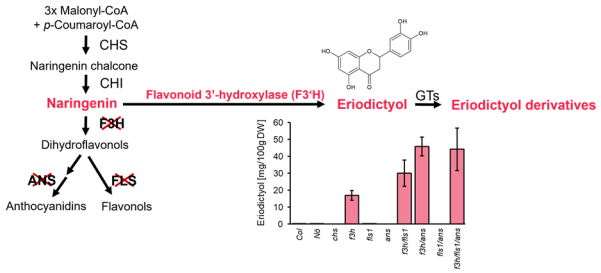


Figure 2: *In planta* eriodictyol derivatives production by targeted knock-out of major flavonoid 2-ODD enzymes. The bypass reaction leading to enhanced accumulation of eriodictyol derivatives in the novel *f3h/fls1/ans* mutant is illustrated. Eriodictyol concentration in siliques was measured by LS-MS (n=4). CHS, Chalcone synthase; CHI, Chalcone isomerase; F3H, Flavanone 3-hydroxylase, FLS, Flavonol Synthase; ANS, Anthocyanidin synthase; GTs, Glycosyltransferases. © H. Schilbert

The identification of these bypass reactions and the production of eriodictyol derivatives hold implications for the development of dietary supplements. Eriodictyol and its derivatives, known for their bitter taste-masking properties, offer promising opportunities for the creation of novel and palatable dietary supplements. This research has recently been published in BMC Plant Biology:

Hanna Marie Schilbert*, Mareike Busche*, Vania Sáez, Andrea Angeli, Bernd Weisshaar, Stefan Martens, and Ralf Stracke (2024). Generation and characterisation of an *Arabidopsis thaliana f3h/fls1/ans* triple mutant that accumulates eriodictyol derivatives. BMC Plant Biology, *shared first authorship

(H. Schilbert, R. Stracke, B. Weisshaar)

The missing link of coenzyme Q biosynthesis identified: role of COQ4 in humans

Coenzyme Q is structurally related to vitamins, but proteobacteria and all respiring eukaryotic cells have to synthesize CoQ. In humans, deficient coenzyme Q_{10} (CoQ_{10}) biosynthesis can have drastic consequences with a wide variety of clinical manifestations. Severe multisystem CoQ_{10} deficiency often results in neonatal or infant death. Mutations in 8 of 13 genes of CoQ_{10} biosynthesis are known. For COQ4, haploinsufficiency causes, e.g., neonatal brain anomalies and epileptic encephalopathy due to CoQ_{10} deficiency. A new publication identified the missing link of CoQ_{10} biosynthesis and revealed which reaction(s) are catalyzed by the enzyme encoded by COQ4.

In 2021, Arthur Burgardt and Volker Wendisch converted a *Corynebacterium glutamicum* that natively lacks CoQ_{10} to a CoQ_{10} overproducer based on the knowledge of the complete CoQ_{10} biosynthesis pathway in *Escherichia coli* (DOI: 10.3389/fbioe.2021.650961). In 2022, *C. glutamicum* CoQ_{10} overproduction was improved (DOI: 10.3390/metabo12050428) in collaboration with Ludovic Pelosi and Fabien Pierrel from Grenoble (first and last authors of the 2024 paper, s. below). This biotech work also helped to provide insight into human CoQ_{10} biosynthesis. Having built up CoQ_{10} biosynthesis in *C. glutamicum* from scratch using heterologous enzymes in a stepwise manner allowed us to interrogate the function of proteins - COQ4 in this example - with presumed activity in CoQ_{10} biosynthesis.

Cooperation partners from Grenoble (France), Padua (Italy), Madison (USA), St. Louis (USA) and Sevilla (Spain) revealed that COQ4 deficient human cells accumulate the intermediate 3-decaprenyl-4-hydroxybenzoic acid ($_{10}$P-HB). The fact that a mutant unable to bind zinc ions due to a single amino acid substitution also accumulated this intermediate led to the hypothesis that COQ4 possesses enzyme activity for conversion of this intermediate towards CoQ_{10} (Figure 3).

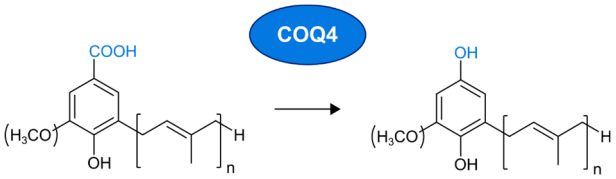


Figure 3: Reaction catalyzed by COQ4. © V. Wendisch

This idea was corroborated by complementing *E. coli* mutants, however, *E. coli* simultaneously synthesizes CoQ_{10} and menaquinone, hampering interpretation of the genetic complementation results. Using the *C. glutamicum* system unequivocally identified COQ4 as C1-hydroxylase and C1-decarboxylase active with $_{10}$P-HB. This study fills a major gap in the knowledge of eukaryotic CoQ_{10} biosynthesis and contributes to understanding the pathophysiology of human primary CoQ_{10} deficiency due to COQ4 mutations.

Pelosi L, Morbiato L, Burgardt A, Tonello F, Bartlett AK, Guerra RM, Ferizhendi KK, Desbats MA, Rascalou B, Marchi M, Vazquez-Fonseca L, Agosto C, Zanotti G, Roger-Margueritat M, Alcázar-Fabra M, García-Corzo L, Sánchez-Cuesta A, Navas P, Brea-Calvo G, Trevisson E, Wendisch VF, Pagliarini DJ, Salviati L, Pierrel F (**2024**). *Molecular Cell, **84**,* 981-989.
DOI: 10.1016/j.molcel.2024.01.003

(A. Burgardt, V. Wendisch)

Winter brewing competition of the Technical Faculty on the 31st of January

After our summer brewing competition, everyone looks forward to the winter brewing competition during the long and cold season. Like every year, the competition took place in the foyer of CeBiTec on January 31, despite the cold outside temperatures. Students in the Molecular Biotechnology degree programme and, increasingly, students from other disciplines are building up their brewing expertise in the tried-and-tested seminar Process Development and Process Management in Modern Brewing. Together with our master brewer Matthes Haese, they then compose their own recipes, whereby there are no limits to their imagination - and no purity laws either. This winter, beers were created that were extremely well received by the audience of over 100 colleagues and the jury of lecturers from the department: the points awarded were very close together, which indicates that the beers were technically produced at a high standard and that in the end, it was the taste that was decisive.

The beer-tasting jury. © D. Cholewa

First place went to Citrusbier, followed by Braukraut with great spicy flavours. At third place came BerryBrew stuffed with real berries followed by Bierbon Vanilla...but can you argue about the flavour? As the 50-litre beer kegs were slowly emptied at around 8 pm and onwards, there is a lot to suggest that every beer was good. The Herforder brewery sponsored a keg as a reference beer for a classic pilsner, as well as all four taps: thank you very much at this point. A big thank you to all those present for their loyalty and a big thank you to CeBiTec for allowing us to organize the brewing competition in this beautiful location in winter.

(D. Cholewa)

Impressum

Centrum für Biotechnologie
Universität Bielefeld
Dr. Lutz Wobbe
Universitätsstr. 27
33615 Bielefeld
Germany

Concept & Idea:
Dr. Stefan Weidner
info@cebitec.uni-bielefeld.de