

CeBiTec – Quarterly

Summer 2021



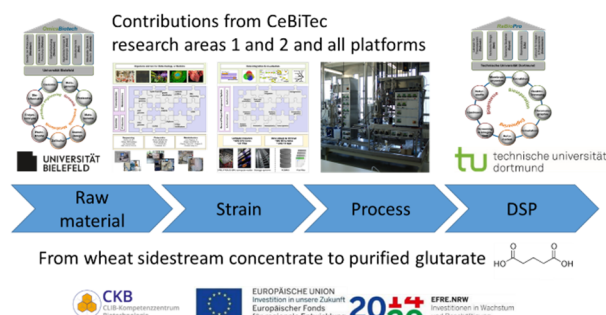
- ▶ Flux enforcement and Adaptive Laboratory Evolution (ALE) accelerate sustainable production of glutarate by *Corynebacterium glutamicum*
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Flux enforcement and Adaptive Laboratory Evolution (ALE) accelerate sustainable production of glutarate by *Corynebacterium glutamicum*

Glutarate is a dicarboxylic acid used, e.g., as monomer for polymers such as biopolyamides or polyesters. Since its chemical synthesis involves hazardous substances such as cyanide, green routes to its production are sought after. For fermentative glutarate production we embedded a five-step pathway extending L-lysine biosynthesis and involving two transamination reactions in a *Corynebacterium glutamicum* L-lysine overproducing strain. Deletion of the gene for the primary ammonium assimilating L-glutamic acid dehydrogenase

coupled growth to glutarate production, a metabolic engineering strategy named flux enforcement. Based on this previous work ([Pérez-García et al. Front Microbiol. 9:2589](#)), Carina Prell and Volker F. Wendisch (Research Area 1) used adaptive laboratory evolution to select for faster growing and due to flux enforcement faster producing strains. Tobias Busche and Christian Rückert (Research Area 2) identified SNPs by genome sequencing. Reverse genetics experiments by Carina Prell allowed to establish causality. Subsequently, glutarate

production was developed at the 2 L bioreactor-scale operated in batch and fed-batch mode in a mineral salts medium. This enabled us to reach a titer of 22.7 g L⁻¹, a yield of 0.23 g g⁻¹ and a volumetric productivity of 0.35 g L⁻¹ h⁻¹. Lea Nolte and Christoph Brandenbusch (Technical University Dortmund) developed downstream processing based on reactive extraction of glutarate from fermentation broth.



This successful collaboration between research areas 1 and 2 of the CeBiTec and with the Technical University of Dortmund was funded by the CLIB Competence Center Biotechnology (speaker: Volker F. Wendisch). Within the CLIB Competence Center Biotechnology, a real-world sustainable feedstock was available: a concentrated sidestream of Germany’s largest starch factory Jäckering. This wheat sidestream concentrate contains the non-starch fraction of wheat grains and was shown by Arthur Burgardt, Carina Prell and Florian Meyer to support production of glutarate as well as its hydroxylated derivative L-2-hydroxyglutarate by recombinant *C. glutamicum* strains.

Prell C, Busche T, Rückert C, Nolte L, Brandenbusch C, Wendisch VF (2021) *Microbial Cell Factories* 20: 97. DOI: [10.1186/s12934-021-01586-3](https://doi.org/10.1186/s12934-021-01586-3)

Prell C, Burgardt A, Meyer F, Wendisch VF (2021) *Frontiers in Bioengineering and Biotechnology* 8: 630476. DOI: [10.3389/fbioe.2020.630476](https://doi.org/10.3389/fbioe.2020.630476)

(V. Wendisch)

Analysis of GABI-Kat T-DNA insertion lines by long read sequencing reveals chromosome rearrangements

We sequenced the genomes of 14 *Arabidopsis thaliana* GABI-Kat T-DNA insertion lines and detected complex T-DNA insertion arrays as well as 11 previously unknown T-DNA insertion sites. Chromosome fusions and translocations were identified as a by-product of T-DNA insertion mutagenesis, including compensating translocations that do not contain T-DNA at the junctions (Fig. 1).

We developed an automated workflow which supports the in-depth analysis of long read data from T-DNA insertion lines. The workflow from DNA extraction to (local) assembly of the T-DNA loci can easily be completed within a week, offering fast access to a complete line characterization including previously undetected insertions. Given that many T-DNA insertion mutants have been used to define gene functions in *A. thaliana*, comprehensive characterization of

relevant alleles with long read sequencing is recommended. Generation of these results at the CeBiTec was made possible by the ONT

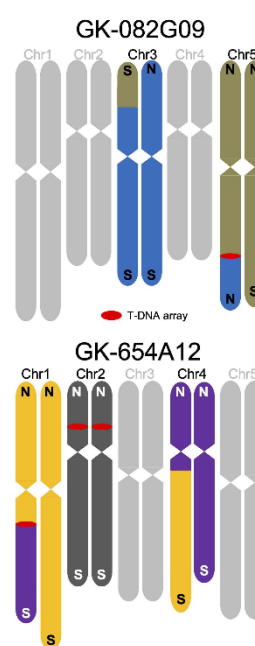


Figure 1 |

Ideograms of the five chromosomes from two example lines.

N, northern end of chromosome;

S, southern end of chromosome.

T-DNA insertions are indicated in red.

sequencing infrastructure established by Jörn Kalinowski, and by the compute power provided by de.NBI cloud resources. The paper entitled "Large scale genomic rearrangements in selected *Arabidopsis thaliana* T-DNA lines are caused by T-DNA insertion mutagenesis" by Pucker, Kleinbölting & Weisshaar has been accepted for publication at BMC Genomics and is available as preprint on bioRxiv.

(<https://doi.org/10.1101/2021.03.03.433755>)

(B. Pucker, N. Kleinbölting & B. Weisshaar)

CeBiTec receives funding from the BMBF for the development of novel RNA production systems in bacteria

The CeBiTec research group Microbial Genomics and Biotechnology, led by Prof. Jörn Kalinowski, received a grant for the development of production of mRNA by fermentation. The grant is worth more than 1.3 million € and shared with the start-up company SenseUP GmbH, led by Dr. Georg Schaumann and located at Forschungszentrum Jülich. The team wants to develop cost-effective and scalable processes for the production of RNA-based active ingredients over the next three years. For this purpose, the Parliamentary State Secretary in the Ministry of Research and Education (BMBF), Thomas Rachel, MdB presented the funding decision in a ceremony at the Forschungszentrum.

RNA-based active ingredients are currently establishing themselves as a new vaccine class that is making a significant contribution to containing the COVID19 pandemic. Many other RNA-based vaccines are in preparation, and RNA-based active ingredients are also of great

importance for the treatment of cancer and serious hereditary diseases. However, they can also be used for biological pest control in agriculture. This makes them an alternative to chemical pesticides or the use of genetically modified plants. Chemical pesticides pollute the soil and groundwater and often have an unspecific effect, in that they also damage beneficial organisms. RNA-based organic pesticides, on the other hand, have a highly selective effect on the plant pests that are to be combated. However, the production of large quantities of RNA-based active ingredients is technically demanding and cost-intensive. For this reason, the CeBiTec researchers are working with SenseUp GmbH, an expert in modern production development, to create production processes that can be used to produce RNA with the help of microorganisms. In this way, the required amounts of RNA can be made available inexpensively for medical as well as agricultural use.



Handover of the funding notification: Dr. Georg Schaumann (SenseUp GmbH), Prof. Dr. Jörn Kalinowski (CeBiTec, Universität Bielefeld) and Thomas Rachel, Parliamentary State Secretary in the BMBF.

(J. Kalinowski)

Recent CeBiTec-project on sustainable fatty nitrile synthesis highlighted by FNR

The recent CeBiTec-project “Biotechnological production process for the industrial production of fatty nitriles from fats, oils and their derivatives”, which was funded by the German Federal Ministry of Food and Agriculture (BMEL) through its project management agency Fachagentur Nachwachsende Rohstoffe e. V. (FNR), has now been highlighted in an official press release of the FNR as a selected successful funding example in the field of sustainable chemistry together with two other projects.

The research area of utilizing biorenewable feedstocks for the production of industrial chemicals gains more and more interest in academia as well as industry. An interesting class of catalysts for this purpose are enzymes. However, a challenge is the development of biocatalytic processes, which are suitable to be operated at high substrate loading, thus leading to high volumetric productivity.

The CeBiTec project focused on a biocatalytic route towards fatty nitriles, which are applied for the production of, e.g., dispersants, emulsifiers and lubricants. The industrial importance of fatty nitriles is illustrated by the fact, that already in 2011 the global fatty nitrile consumption reached 800,000 metric tons. As an alternative to existing routes towards fatty nitriles, the process of the CeBiTec scientists from the Chair of Industrial Organic Chemistry and Biotechnology is based on a two-step chemoenzymatic synthesis starting from fatty aldehydes being readily accessible from biorenewable raw materials. In the initial step,

the corresponding aldoxime is formed via condensation with hydroxylamine, and the subsequent dehydration with an aldoxime dehydratase as a biocatalyst then furnishes the desired fatty nitrile. It is noteworthy that this biotransformation runs at a very high substrate loading, which makes this process interesting for industrial applications. For example, at 1.4 Kg of substrate per liter of reaction medium, a conversion of 93% to the desired *n*-octannitrile was achieved. At a somewhat lower, but still high substrate loading of 655 g/L, a yield of 97.5% was obtained. Furthermore, the reaction is conducted under solvent-free conditions.

Original citation of press release (in German language): FNR, “Grüne Chemie: Neue, effiziente Verfahren für biobasierte Fett- und Acrylnitrile und Polyamide entwickelt”, 2021-04-23, [link](#)

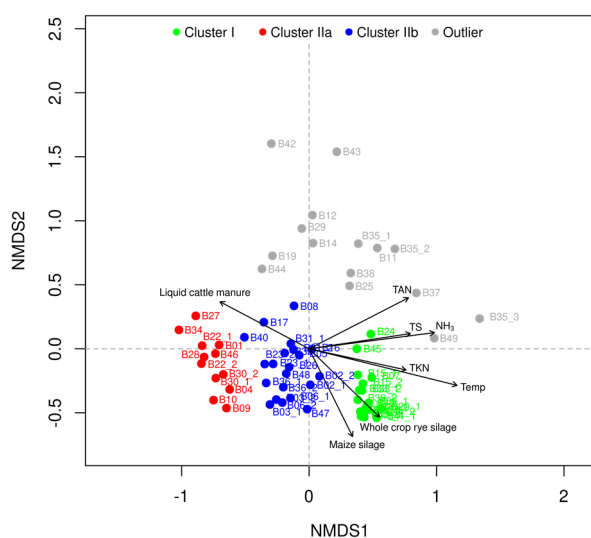
(H. Gröger)

Large-scale German-wide profiling of microbiomes from agricultural biogas plants

The research group ‘Genome Research of Industrial Microorganisms’ headed by Alfred Pühler contributed to the recently completed Biogas Monitoring Program III (BMP-III) funded by the ‘Fachagentur Nachwachsende Rohstoffe e.V.’ (FNR, Federal Ministry of Food and Agriculture). The BMP-III project was carried out in cooperation with partners from the Leibniz-Institute for Agricultural Engineering Potsdam Bornim and the Otto-von-Guericke University in Magdeburg to analyze the complex and dynamic microbiomes involved in anaerobic digestion of organic biomass to biogas within agricultural biogas plants. Project partners at the CeBiTec focused on the analysis of biogas microbiomes

by applying methods of metagenome research, in particular taxonomic profiling using 16S rRNA gene amplicon sequencing. Main results of the project were now [published](#) in the scientific journal ‘Microorganisms’ (MDPI) by Julia Hassa and coauthors.

In the future, German agricultural biogas plants have to be much more flexible regarding their input materials, due to the change from the primary usage of energy crops, such as maize silage, to a residue-based biogas production. This will have a big impact on the biogas microbiomes, since they will be exposed to frequently varying process conditions. In order to still ensure a stable biogas process, a better understanding of the biogas microbiomes and how they respond to management measures is necessary. Accordingly, in the study carried out at the CeBiTec, the microbiomes of 67 full-scale anaerobic digesters of 49 agricultural biogas plants were taxonomically investigated and analyzed.



Non-metric multidimensional scaling (NMDS) of the analyzed 67 biogas microbiomes indicating the process specific clusters I, IIa, IIb and the group of outliers. The vectors show the process parameters, which have the greatest impact on the microbiome structures (Hassa *et al.*, 2021).

It appeared that the taxonomic profiles of the analyzed microbiomes can be grouped into three distinct clusters: I, IIa, IIb and a group of outliers. These clusters are characterized by indicative taxa, which are specific for the process conditions associated with the respective cluster. Findings from these investigations can be used to recognize unfavorable process conditions by prior changes in the microbiome structure.

Especially, monitoring of the indicative taxa should enable early insights into changing process conditions. In the future, biogas process-specific indicative taxa may be used for the development of detection systems for monitoring purposes or advanced biogas process models.

Hassa, J., Klang, J., Benndorf, D., Pohl, M., Hülsemann, B., Mächtig, T., Effenberger, M., Pühler, A., Schlüter, A., & Theuerl, S. (2021). Indicative Marker Microbiome Structures Deduced from the Taxonomic Inventory of 67 Full-Scale Anaerobic Digesters of 49 Agricultural Biogas Plants. *Microorganisms*, 9 (7), 1457.

<https://doi.org/10.3390/microorganisms9071457>

(J. Hassa, A. Schlüter & A. Pühler)

National research data infrastructure for microbiological research

The Bielefeld Institute for Bioinformatics Infrastructure (BIBI) and the Centrum for Biotechnology (CeBiTec) at Bielefeld University are expanding their participation in the National Research Data Infrastructure (NFDI) initiative.

The German Research Foundation (DFG) will fund the NFDI4Microbiota project and grant a total of 15.7 mio EUR to Bielefeld University and nine co-applicants.



NFDI4Microbiota supports microbiological research in Germany with access to data, analytical tools, standards for data and metadata, as well as with a comprehensive training program. The project is coordinated by the Information Center for Life Sciences (ZB MED) in Cologne.

“We are very pleased that, following the proposal for funding by the expert committee in May, the Joint Science Conference has now also given their positive vote. Over the next five years we want to significantly improve the data infrastructure and thus the research conditions for microbiota in Germany,” says bioinformatics scientist Professor Dr. Alexander Sczyrba. He is one of the future project leaders of NFDI4Microbiota.

Microbiota - also known as microbiomes - are communities of microorganisms such as bacteria and fungi. The interaction of microbes in these communities creates effects that can be used, for example, in research fields such as medicine or crop sciences. Within the project, researchers of BIBI and CeBiTec are responsible for the technical infrastructure, a core competence of the institutes, says Sczyrba: “As part of the German Network for Bioinformatics Infrastructure (de.NBI), together with seven other cloud sites across Germany, we already provide a cloud computing infrastructure to life scientists for their research projects.

Within NFDI4Microbiota we are contributing our expertise not only in infrastructure services but also in bioinformatics tools and workflows.”

Researchers of NFDI4Microbiota also want to use

synergies with the ongoing NFDI project NFDI4BioDiversity.

“The topics of biodiversity and microbiota fit together very well in terms of content, so we assume that the colleagues in the NFDI4Microbiota project can benefit from our previous findings,” says genome informatics scientist Professor Dr. Jens Stoye.

He is, alongside Alexander Sczyrba, responsible for NFDI4Microbiota. On the part of Bielefeld University, he also heads the NFDI4BioDiversity project. “We are convinced that with both NFDI projects we will be able to create significant added value for life scientists in the coming years through uniform research infrastructures,” says Stoye. NFDI4BioDiversity is concerned with collecting research data on biological diversity and enabling standardized access. For example, the project aims to provide a data infrastructure for those researchers who deal with the genetic or phenotypic diversity of organisms or the diversity of populations and entire ecosystems.

Research data is often stored only locally and temporarily. This causes data treasures to be lost. The National Research Data Infrastructure (NFDI) aims at changing that: It should be a knowledge repository for the entire research landscape. To this end, it is to establish and further develop a joint research data management system in Germany, for which the various research institutions work together. The federal infrastructure program for research data, standards, survey, analysis and archiving is under the auspices of the German Research Foundation (DFG).

NFDI4BioDiversity was selected in the first funding round 2020 with eight further projects, NFDI4Microbiota with a total of nine further projects now in the second. As in the first round

of funding, the DFG's recommendation was based on a multi-stage, science-driven process in which numerous international reviewers were involved.

(A. Sczyrba & J. Stoye)

Prof. Alfred Pühler`s citation record reaches the impressive number of 50,000 citations

Recently, Prof. Alfred Pühler`s citation record at [Google Scholar](#) reached the impressive number of 50,000 citations. He is therefore by far the most frequently cited researcher at Bielefeld University and his record exceeds those of other

highly cited researchers at Bielefeld University by more than 20,000 citations. His most highly-cited publication was cited more than 7,000 times and published in 1983, describing a method for random transposon insertion mutagenesis in gram negative bacteria.

(B. Weisshaar & L. Wobbe)

TITEL	ZITIERT VON	JAHR
A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria R Simon, U Priefer, A Pühler Bio/technology 1 (9), 784-791	7630	1983
Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium ... A Schäfer, A Tauch, W Jäger, J Kalinowski, G Thierbach, A Pühler Gene 145 (1), 69-73	2582	1994

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