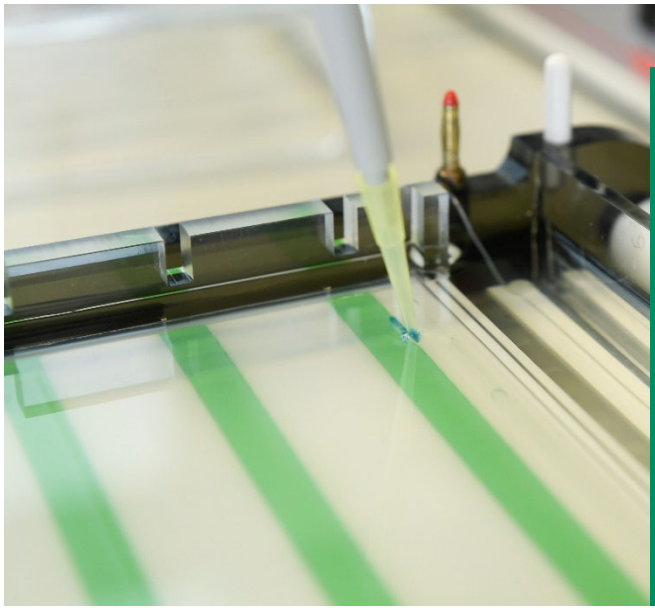
UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

CeBiTec – Quarterly

Winter 2020/2021



- Permanent continuation of the de.NBI network through integration into the Helmholtz Association
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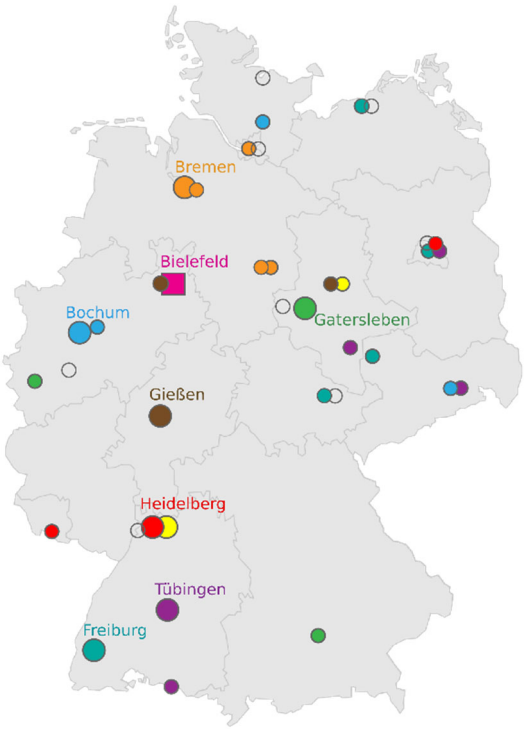
Permanent continuation of the de.NBI network through integration into the Helmholtz Association

For more than five years the German Network for Bioinformatics Infrastructure (de.NBI) has been funded by the Federal Ministry of Education and Research (BMBF). The de.NBI network represents the home of 40 infrastructure projects that are organized in eight so-called service centers. Bielefeld University is involved in one of these service centers. An administration office located at the Center for Biotechnology (CeBiTec) of Bielefeld University coordinates the network. The mission of the de.NBI network is to support life scientists in the analysis of large amounts of data. To this end, Service, Training and Compute areas have been established by the de.NBI network. The Service area provides about 150 software solutions for the analysis of life science data sets.



Geographical distribution of de.NBI service centers (large circles) and the de.NBI Administration Office (square). Individual projects (small circles) associated with service centers are shown.

In the Training area, the handling of these software tools and the results obtained with them are taught. Finally, in the Compute area, a federated cloud has been established at six German sites – one of which is Bielefeld University – enabling users to analyze life science data free of charge in a cloud environment.

The de.NBI network successfully passed its first practical test in times of the Corona pandemic. Without delay, extensive data sets have been analyzed for numerous COVID-19 research projects.

The establishment of a bioinformatics infrastructure in Germany has always been associated with the question of how to make the network's infrastructure permanent. Therefore, the two de.NBI coordinators, A. Pühler and A. Tauch, have been working intensively on this continuation task in recent years. The BMBF indicated that a "political approach" seems to be a valuable strategy to solve the continuation issue. Thereupon the rector and the prorector of Bielefeld University, G. Sagerer and M. Egelhaaf, together with the de.NBI coordinator A. Pühler agreed to discuss the continuation of the de.NBI network with regional members of the German parliament. This approach was crowned with immediate success. The chairman of the CDU/CSU parliamentary group in the Bundestag, R. Brinkhaus, was able to initiate the permanent continuation of the de.NBI network and published a press release. This press release was complemented by a statement of the rector of Bielefeld University, G. Sagerer.

Beginning with January 2022, the de.NBI network should be affiliated with the Forschungszentrum Jülich. How this affiliation will be organized in detail has to be decided in the current year. In the press release of R. Brinkhaus, a new Helmholtz Institute to be founded at Bielefeld University has been proposed.

(A. Pühler)

CeBiTec member and highly cited researcher Karl-Josef Dietz has been elected president of the German Life Science Association (VBIO)

In November 2020 the federal delegate meeting of the German Life Science Association (VBIO, in German "Verband Biologie, Biowissenschaften und Biomedizin") elected the Bielefeld-based plant researcher and CeBiTec member Karl-Josef Dietz as the association`s new president for a period of two years. As an umbrella organization, VBIO is committed to continuously improving the research conditions for life scientists, supporting the dialog between science and the public as well as passing on life scientist`s requests to political consultants. Another concern of VBIO is to foster education, training and professions in biology as well as biomedicine. Here, the transitions should be optimized.

Since 1997 Karl-Josef Dietz holds a chair for "Biochemistry and Physiology of Plants" at Bielefeld University and in November 2020 he was listed for the fifth time as a Highly Cited Researcher in the research field "Plant and Animal Science", an award he also achieved in the years 2015-2018.

The list of highly cited researchers is annually published by Clarivate Analytics and based on the evaluations of the Web of Science and re-citations, which were achieved by the researcher`s publications in past years. The list of Highly Cited researchers comprises about 0.1% of researchers around the world, covering 21 research fields and an additional multidisciplinary field.

(L. Wobbe)

CeBiTec contribution to the banana genome hub

Although Bielefeld is not a natural habitat of banana (*Musa acuminata*) plants, there are several different accessions growing in the greenhouse. Since the reference genome sequence (Pahang) is based on a double haploid accession, we (Genetics & Genomics of Plants, AG Weisshaar) decided to investigate the commonly consumed banana accession 'Dwarf Cavendish' by high-throughput Illumina sequencing.

Fig. 1: Banana plants in greenhouse.

The comparison against the reference accession Pahang revealed a duplication of a large segment of chromosome 2[1]. Following the open science policy of Bielefeld University, the underlying data have been made publicly available in general repositories including a Data Publication[2] available via a registered DOI (Digital Object Identifier) provided by PUB (Publications at the University of Bielefeld).

Fig. 2: Image provided by Mathieu Rouard (The Alliance of Biodiversity and CIAT).
https://banana-genome-hub.southgreen.fr/

Additionally, our sequencing data are shared at the banana genome hub. The CeBiTec dataset is presented in a genome browser next to re-sequencing data contributed by many renowned international institutes (Fig. 2). Details about the latest updates of the banana sequencing datasets are available at the banana genome hub[3].

(M. Busche, B. Pucker, R. Stracke & B. Weisshaar)

References

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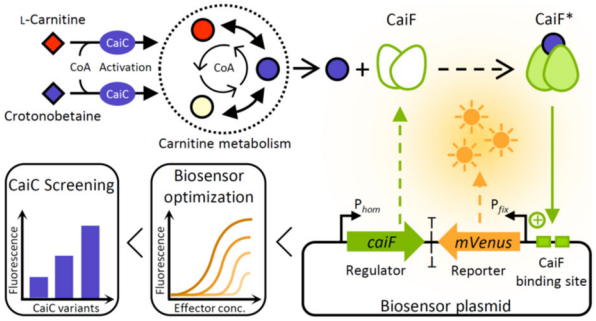
[2] https://pub.uni-bielefeld.de/record/2936278

[3] https://banana-genome-hub.southgreen.fr/content/release-notes

A Biosensor for L-Carnitine in *E. coli*



Strategy and workflow of biosensor development

L-Carnitine is a quaternary amine essential for the intermediary metabolism of eukaryotes by playing an important role in energy production and fatty acid metabolism. Applications can be found in numerous medical fields, for example, in the treatment of cardiovascular diseases and as a dietary supplement to improve weight management and exercise performance. For *E. coli*, L-carnitine is important as an osmolyte and as terminal electron acceptor for anaerobic respiration. The reversible reduction of carnitine to γ-butyrabetaine has been employed for the aerobic production of L-carnitine by *E. coli* in several studies and in industry. However, L-carnitine metabolism, which is dependent on

the *cai* and *fix* operons, and its regulation have not been fully elucidated. For example, the co-activator of the transcriptional activator protein CaiF remained elusive. Pierre Kugler, Deborah Fröhlich and Prof. Dr. Volker F. Wendisch (CeBiTec and Faculty of Biology) analyzed expression of a transcriptional fusion of the cognate *fix* promoter, including its CaiF binding sites, to a promoter-less fluorescent reporter gene. Genetic loss-of-function and gain-of-function experiments demonstrated that crotonobetainyl-CoA, an intermediate of L-carnitine metabolism, is essential for activation of the *cai* and *fix* operons by CaiF. This prompted them to propose crotonobetainyl-CoA as the sought coactivator of CaiF. Based on these findings, a dual-input biosensor for L-carnitine and crotonobetaine was developed and further optimized by adapting the culture medium and engineering the host strain. The genetically encoded biosensor system was able to report external L-carnitine concentrations in the range of 0.1–100 µM with ~50-fold induction. Moreover, it supported detection of crotonobetaine with ~60-fold induction in the same concentration range. As an application of the biosensor, putative homologues of the betaine:CoA ligase CaiC, which catalyzes the first step of carnitine reduction, from *Citrobacter freundii*, *Proteus mirabilis*, and *Arcobacter marinus*, were screened and shown to be functionally active CaiC variants. This confirmed that the enzyme is conserved not only in the order *Enterobacterales*, but also in ε-proteobacteria. In the future, the new CaiC variants and the constructed biosensor will be valuable for the development of microbial cell factories for sustainable production of L-carnitine.

(P. Kugler & V. Wendisch)

References

Kugler, P., Fröhlich, D., & Wendisch, V. F. (2020). *ACS Synthetic Biology*, *9*(9), 2460-2471. DOI: 10.1021/acssynbio.0c00234

Establishing microalgae as functional food and feed

The **Algae Biotechnology & Bioenergy Group** (Prof. Dr. Olaf Kruse & Dr. Viktor Klassen) and **Fermentation Technology Group** (Dr. Dominik Cholewa & Dr. Joe Max Risse) of the **Center for Biotechnology at Bielefeld University (CeBiTec)** in collaboration with two industrial partners **Microganic GmbH** and **Mack bio-agrar GmbH** have successfully acquired the R&D project "AlgaSubst" funded by ZIM (Central Innovation Programme of the German Federal Ministry of Economy & Energy for small and medium-sized enterprises (SMEs)).

The "AlgaSubst" consortium

The framework of "AlgaSubst" aims at developing microalgae products that meet the growing demand for plant-based functional foods with a customised ingredient profile. This ambitious goal shall be achieved by applying GMO-free laboratory evolution setups with advanced single cell screening methods **(Algae Biotechnology & Bioenergy Group)** and an innovative fermentation technology for heterotrophic cultivation **(Fermentation Technology Group)**. Furthermore, the raw materials used for the cultivation of microalgae

should comply with sustainability and biological aspects **(Mack bio-agrar GmbH)** and should ensure wide target group coverage within the human and animal food industry through improved appearance, taste and stability **(Microganic GmbH)**. The two CeBiTec partners are funded with 400.000 € for a period of 2 years. (V. Klassen & O. Kruse)

High cell density cultivation for sustainable, recombinant cadaverine production in the microalga *Chlamydomonas reinhardtii*

The chemical industry is currently switching to renewable feedstocks for material and energy use through bio-production of key base chemicals. The EFRE-funded CKB-project, coordinated by CeBiTec member Prof. Dr. Volker Wendisch aims at accelerating this process, utilizing bacterial and microalgal production hosts. In a recent report, published in *Bioresource Technology*, members of the Algae Biotechnology & Bioenergy group (led by Prof. Dr. Kruse), demonstrate the first successful engineering of the green microalga *Chlamydomonas reinhardtii* for bio-production of the non-native polyamine cadaverine (Fig. 1). Cadaverine is a versatile precursor and building block for bio-polyamide synthesis. The derived bio-plastics have excellent material properties and a broad range of potential applications as engineering (automotive and electronics) and medicinal plastics, textile fibers, as well as films and coatings.

Recombinant bio-production of cadaverine can be achieved via enzymatic decarboxylation of the endogenous amino acid lysine. *C. reinhardtii* is a promising production host, as it combines fast and inexpensive phototrophic growth with advanced options for genetic engineering. Two bacterial lysine decarboxylases were overexpressed and targeted to different compartments of the microalgal cell to establish initial cadaverine production.

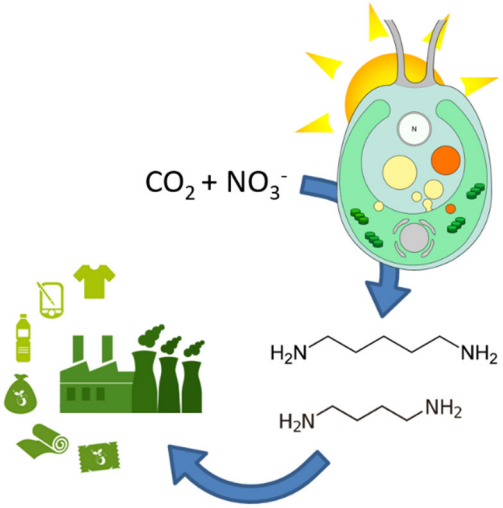


Fig. 1: Microalgal bio-production of cadaverine from CO_2 and nitrate

This production process was further maximized with the optimization of cultivation conditions, necessitated by the poor performance of available cultivation media. They were developed for lab-scale settings and support only low cell densities, making them unsuitable for large scale, industrial production processes. To overcome these limitations, a new, innovative strategy for fully phototrophic and easy-to apply high cell density cultivations was developed. By systematically increasing macro- and micronutrients in combination with elevated light and CO_2, the team was able to achieve a 6-fold increase in cell number and biomass accumulation, reaching up to ~$2 \cdot 10^8$ cells/mL and 20 g/L biomass dry weight, respectively. Applying high cell density cultivations in different photobioreactors

increased final cadaverine yields, accordingly, to 0.24 g/L after 9 days and a maximum productivity of 0.1 g/L/d. Biotechnological application of *C. reinhardtii* as a recombinant production host will greatly benefit from this optimized cultivation strategy.

(R. Freudenberg)

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Investitionen in Wachstum und Beschäftigung
EUROPÄISCHE UNION
Investition in unsere Zukunft
Europäischer Fonds für regionale Entwicklung

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Genomic SARS-CoV-2 surveillance at the Technology Platform Genomics

In these days, the COVID-19 pandemic is the most important challenge to society. Although the political institutions have taken various strategies to reduce infections, it now becomes clear that the SARS-CoV-2 virus had too many chances to mutate into variants with increased fitness. This fitness either results in a higher infection rate or, even more serious, in variants that re-infect COVID-19 patients, pointing to a certain amount of immune escape.

An example of the first kind is the variant B.1.1.7, first detected in the UK. Meanwhile, it is clear that it harbours a number of mutations that, among other gene products, affect the so-called spike protein, the contact point to the cellular ACE2 receptor [1]. It has been shown, that the B.1.1.7 variant of the virus has a mutated spike protein (D614G) that is more efficient in contacting the ACE2 receptor and therefore shows an increase in infectivity by around 35%. This higher infectivity has putatively led to a huge increase in cases in the UK despite of lockdown measures and is currently also suspected to overwhelm the health care system in Portugal. Besides, there are indications of a higher mortality rate induced by B.1.1.7 variant, probably due to the fact, that it can infect more cells in the human body and the virus load is increased.

Unfortunately, the UK variant is not the only virus “variant of concern” (VOC). As expected from virus evolution, immune escape mutants are strongly favoured in highly infected populations. Therefore, two virus variants, one from South Africa (B.1.351) and one from Brazil (P.1) have been shown to lead to a varying interaction with sera from COVID-19 patients, indicating a partial immune escape [2].

In addition, an interplay with animal reservoirs can lead to a certain amount of virus evolution, creating variants with re-infection potential [3]. The most dramatic one to date was the infection of humans by minks in fur farms in Denmark. An infected human originally transferred the respective virus to minks, it subsequently evolved in minks and accumulated spike protein mutations, also making it less amenable by the human immune system of COVID-19 patients.

These examples clearly show the utmost importance of a genomic surveillance, using coronavirus genome sequences for judging on containment and quarantine measures. We have been sequencing SARS-CoV-2 variants from the Bielefeld region since March 2020. The main scientific projects pursued are:

CoV2Bi: Coronavirus surveillance in the Bielefeld region. Together with clinicians from the Ev.

Klinikum Bethel (Dr. Christiane Scherer) and a local testing lab (MVZ Diamedis, Sennestadt), we followed virus signatures between March and June 2020. We could show that several small infection clusters in Bethel have been efficiently contained, validated by the fact that these virus mutation signatures never reappeared in databases after this time. Also, the large outbreak in a nearby meat factory was analysed and it became clear that the virus did not come from other countries, but had a kind of "Westfalian" signature.

CoV2Fly: Coronavirus surveillance at Dortmund airport. In cooperation with a testing lab in Dortmund (MVZ Eberhard und Partner), we analysed samples taken from returning passengers that provided samples in a voluntary fashion. We could show that the mutation signatures clearly reflect the departing region and some signatures indicated transfer flights from larger airports. It became evident that the summer vacation time had a clear mixing effect among locally evolved virus variants.

In the course of airport surveillance, the last flight from London-Stansted to Dortmund on the 20th of December 2020 was of special importance. Due to a mandatory SARS-CoV-2 testing, seven infected cases were detected among 100 passengers and by sequencing, we found that three cases had the B.1.1.7 signature.

Currently, there is a huge interest in genomic coronavirus surveillance and a novel ordinance now requests that 5% of all positive samples now have to be sequenced. Therefore, these efforts will continue in the CeBiTec, especially because the scientific experts agree that more than these 5% need to be sequenced in order to closely monitor the spread of the known and future VOCs.

Again, it is clear that the vaccination campaign, especially if its speed is not highly increased, will give rise to even more immune escape variants in the near future. It is our mission, to put all available resources into the genomic surveillance, especially in highly vaccinated environments. This project will be conducted together with the two large Bielefeld clinics and the consortium will apply for funding.

(Jörn Kalinowski and the CeBiTec Coronavirus Surveillance team*)

*The CeBiTec Coronavirus Surveillance team:

David Brand, Tobias Busche, Markus Haak, Levin-Joe Klages, Marina Simunovic, Alexander Sczyrba and Svenja Vinke

References

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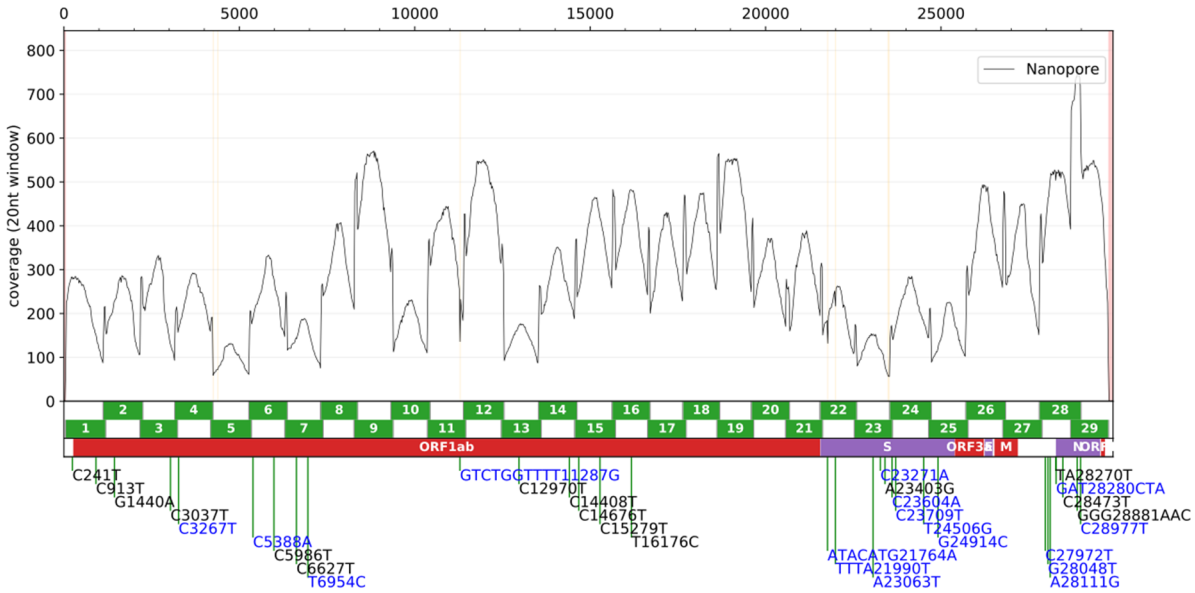

Image of our in-house virus sequencing software solution CoV2Seq, which shows the results of an amplicon sequencing experiment using the nanopore sequencing technology. From top to bottom: read coverage, annotation of amplicons, virus genome annotation, and mutation calls. It shows one of the detected B.1.1.7 variants.

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