ERA CoBioTech

BioTech Research & Innovation Hack

2021

ERA CoBioTech Funded Projects at A Glance: MERIT

MicroalgaE as Renewable Innovative green cell facTories

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MERIT is an international research initiative comprising groups from four different countries and an industrial partner to engineer eukaryotic microalgae for high-value, functionalized diterpenoid production and for the development of associated cultivation technologies.

A novel synthetic biology platform to synthesize various diterpenoids in microalgae from carbon dioxide with an improved partitioning of fixed carbon into desired products

This project will leverage state of the art synthetic biology techniques to engineer microalgae for the sustainable production of high-value, industrially relevant diterpenoid products from carbon dioxide and light. Twenty carbon atoms containing diterpenes are complex, often oxy-functionalized secondary metabolites found largely in plants. Their variety and complexity have made them incredibly interesting for numerous applications as medicines, antimicrobial agents, and high-value chemicals. The complex structures of diterpenoids are difficult and costly to chemically synthesize and can be expensive or inefficient to purify from their native host organisms. Heterologous expression of modular terpene synthase pathways can be used to produce non-native terpenoids. Microalgae are naturally optimized to produce GGPP as the precursor for light harvesting and photoprotective pigments. Over-expression of diterpene synthases (DiTPS) allows the conversion into the numerous diterpenoid products. Algae hold the additional benefit of rapid growth rates in simple mineral salt solutions using only light and CO_2 as energy inputs. These organisms are ideal hosts to produce diterpenes and are inherently sustainable production chassis.

Advanced genetic engineering of two microalgal strains for efficient diterpenoid production

Multiple levels of strain engineering and synthetic biology will be implemented to create innovative green cell factories with enhanced carbon flow from CO_2 to terpenoids. A publically accessible library of characterized genetic parts for nuclear transformations of two promising algal strains (C. reinhardtii, P. tricornutum) will be designed to enable Build-Design-Test-Learn-Cycles for combinatorial expression of DiTPSs. Various constructs will be created to produce industrial-relevant and novel 'new-to-nature' diterpenoid products with incredible potential for numerous biotechnological applications. Advanced metabolic engineering will further increase channeling of fixed CO_2 into the desired diterpene product and improve the overall yield. Optimized strains will be grown to scale and processes for diterpene product extraction will be designed. Diterpenoid production and extraction will be demonstrated in an industrial-relevant environment at TRL_5 and is rendered by a techno-economic model and LCA to assist integrated bioprocess design.

Main results

The research consortium developed two genetic tool kits based on the MoClo syntax for the efficient production of diterpenoids from the algal cell and provides a comprehensive performance analysis. All parts will be shared with the research communities via established distribution platforms (Chlamy Resource Center). Combinatorial expression of DiTPSs lead to the initial production of diterpenoids and yields were further improved by co-expression of metabolically active enzymes increasing the substrate availability (GGPP by co-expression of SpDXS and CrGGPP) and an iterative increase in enzyme copy number. Sophisticated cultivations under controlled lab scale conditions led to the establishment of a high cell density cultivation and the fully phototrophic production of 224 mg/L sclareol in C. reinhardtii. Our results show, that green microalgae hold incredible potentials in MEP-Pathway activities and carbon flux towards heterologous diterpenoid production. An initial production of 5 mg/L manoyl oxide in P. tricornutum was achieved while engineering is still ongoing. Detailed cultivations and extractions at scale are currently performed and demonstrate feasible production in an industrial-relevant technology. An innovative extraction method using hollow fiber membrane assists in the gentle extraction of production without affecting biomass accumulation.

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Consortium:

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Project duration:

1 August 2018 - 31 January 2022

Total budget: 1.7 €M

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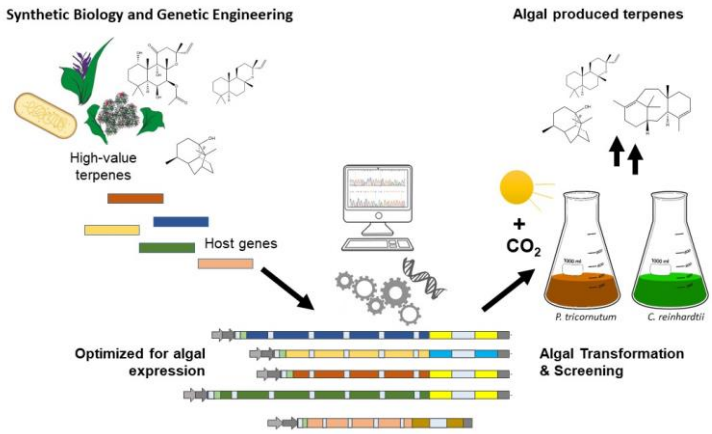

Figure 1: Efficient heterologous terpenoid in industrially relevant microalgae

Diterpenoid biosynthesis pathways discovered in plants has been implemented in engineered microalgae to design innovative cell factories for the high value bio-production and development of a European bio-economy.

Limited nuclear transgene expression due the lack of strong expression elements prevent development of C. reinhardtii towards a biotechnological host for industrial-relevant applications. Researchers at the Center for Biotechnology (CeBiTec) from Bielefeld University systematically investigated several endo- and exogenous introns and identified multiple sequences, which elicit strong IME in C. reinhardtii. Insertion into coding sequences were found to positively affect expression through a synergy of additive transcription enhancement and exon length reduction. Stimulating Introns were also found to be broadly applicable in different expression constructs and induced high protein accumulation in this alga.

- Baier T, Jacobebbinghaus N, Einhaus A, Lauersen KJ, Kruse O. Introns mediate post-transcriptional enhancement of nuclear gene expression in the green microalga Chlamydomonas reinhardtii. PLoS Genet. 2020. doi: 10.1371/journal.pgen.1008944

Project members also designed a new, synthetic algal promoter for efficient transgene expression. By systematically evaluating existing expression elements, combined with rational promoter engineering strategies, Einhaus et al. established novel, synthetic expression elements and improved the standardized application of synthetic biology tools. Additionally, Geisler et al. exploited the impact of terminators on transgene expression.

- Einhaus A, Baier T, Rosenstengel M, Freudenberg RA, Kruse O. Rational Promoter Engineering Enables Robust Terpene Production in Microalgae. ACS Synth Biol. 2021. doi: 10.1021/acssynbio.0c00632.
- Geisler, Katrin, Mark A. Scaife, Paweł M. Mordaka, Andre Holzer, Eleanor V. Tomsett, Payam Mehrshahi, Gonzalo I. Mendoza Ochoa, and Alison G. Smith. "Exploring the Impact of Terminators on Transgene Expression in Chlamydomonas reinhardtii with a Synthetic Biology Approach" Life 2021. https://doi.org/10.3390/life11090964

Future prospect

Further strain engineering of C. reinhardtii and new, advanced genetic strategies to establish high-level transgene expression are crucial steps to establish microalgae as sophisticated green cell factories. Combined with new cultivations strategies to enable high cell density under controlled phototrophic as well as heterotrophic conditions, microalgae can compete with traditional, fermentative hosts.
Our results demonstrate the vast potential of microalgae as promising and sustainable hosts for high value bio-production. As stakeholders are regularly invited to progress meetings and project discussions, the project generates many new avenues of commercialization potential and significantly contribute to the development of the European bio-economy.

Website: https://merit-project.net/

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