MicroalgaE as Renewable Innovative green cell facTories (MERIT)

Olaf Kruse[1], Alison Smith[2], Rene Wijffels[3], Josue Heinrich[4], Andrew Spicer[5]

[1]Bielefeld University / Center for Biotechnology, Bielefeld, Germany

[2]University of Cambridge / Plant Sciences, Cambridge, United Kingdom

[3]Wageningen University / Bioprocess Engineering, Wageningen, Netherlands

[4]Universidad Nacional del Litoral / Facultad de Bioquímica y Ciencias Bológicas, Santa Fe, Argentina

[5]Spicer Consulting Limited / Algenuity, Stewartby, United Kingdom

This project [MERIT] will leverage state of the art synthetic biology techniques to engineer microalgae for the sustainable production of high-value, medically and industrially relevant novel diterpenoid products from carbon dioxide and light. Twenty carbon containing (C20) 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. All organisms produce the same 5-carbon building blocks used in terpenoid production pathways. Heterologous expression of modular terpene synthase pathways can be used to produce non-native terpenoids in engineered hosts. Production of terpenoid products in fermentative hosts has become a mature technology, however, relies on unsustainable use of organic carbon sources such as glucose and inherently competes for agricultural resources. Fermentative microbial hosts are additionally not optimized for the production of the C20 diterpenoid precursor geranylgeranyl pyrophosphate (GGPP). Microalgae, however, are naturally optimized to produce GGPP as the precursor for light harvesting and photoprotective pigments in the cell. Over-expression of diterpene synthases (DiTPS) allows the conversion of this precursor into the numerous carbon skeletons of diterpenoid products. Algal cells also represent ideal chassis for the expression of cytochrome P450 monooxygenases (CYPs), which are needed for oxy-functionalization of diterpene backbones to specialized structures. In photosynthetic cells, CYPs can be coupled to photosynthetic electron transport chains for efficient redox potential, an otherwise limiting factor in non-photosynthetic hosts. Combination of DiTPS and CYPs in an algal chloroplast is the ideal chassis for complex oxy-functionalized

diterpenoid production, where both high concentrations of precursor and the redox electron flow are abundant. 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 for the production of diterpenes and are inherently sustainable production chassis. To date, engineering of microalgae for robust expression of transgenes has been a major limiting factor to their widespread application as green-cell factories for complex biotechnological targets. Through combined efforts of strain domestication and synthetic biology, the development of synthetic eukaryotic algal transgenes (SEATs) have recently been demonstrated to facilitate advanced engineering of these highly promising organisms.

In this project, an international team of experts in algal synthetic biology, cultivation, and industrial process modelling, will join forces to design optimized algal strains and the accompanying industrial production as well as product extraction processes for light-driven conversion of CO_2 into high-value diterpenoid products. This collaborative team represents global leaders in algal synthetic biology, outdoor algal cultivation, photobioreactor design, and process modelling. The MERIT team already successfully engineered pathways for the production of several diterpenoids in microalgae. Multiple levels of strain engineering and synthetic biology will be implemented to create green cell-factories with enhanced carbon flow from CO_2 to terpenoids. Various DiTPS and CYPs will be combined to produce novel ‘new-to-nature’ diterpenoid products with incredible potential for numerous applications. Optimized strains will be grown to scale and processes for diterpene product extraction designed. The project will generate many new avenues of commercialization potential and significantly contribute to the development of the European Bioeconomy.