Department of Green Technology

Uncovering the algal biochemistry to engineer compartmentalized production of hetelorogous high value terpenoids

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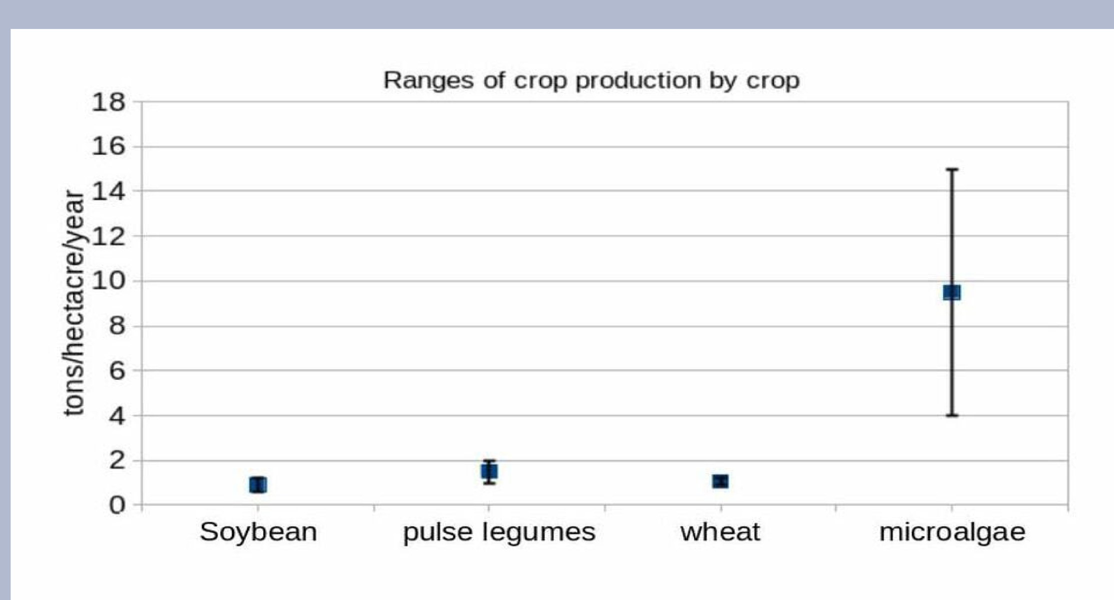
Introduction

Microalgae – Sustainable Producers…

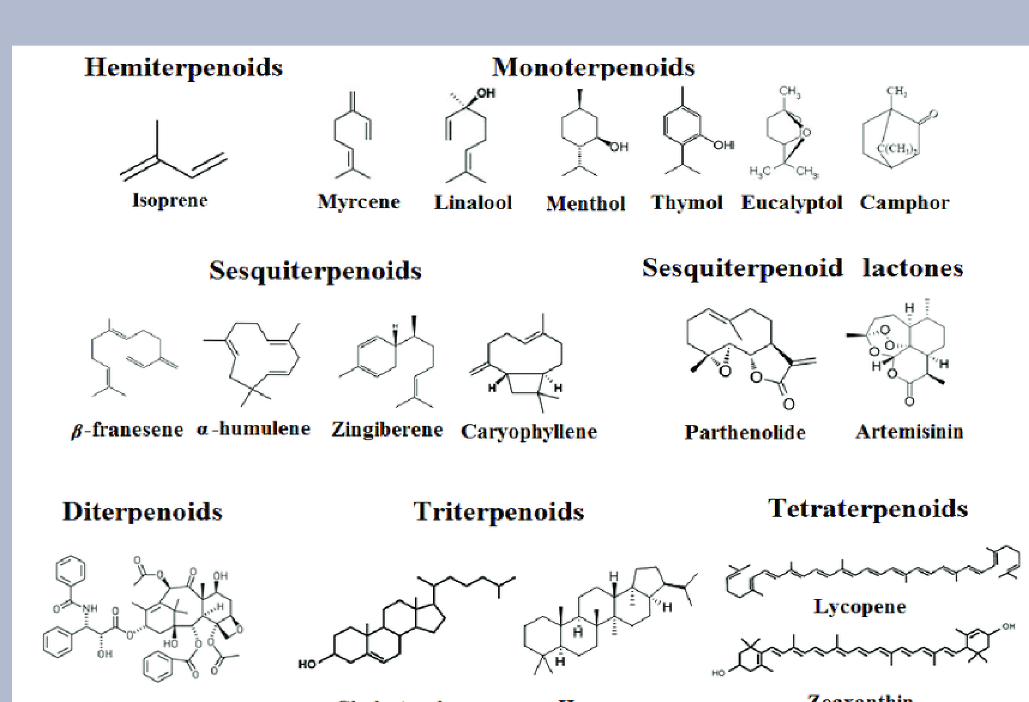
Microalgae can directly convert sunlight and CO2 into biomass and valuable products. They are able to grow in sea- or wastewater, while having much higher growth rates then plants. In respect to those aspects, microalgae pose great potential as a sustainable production platform. As terpenoids are the biggest group of metabolites on the market, and photosynthetic organisms produce naturally the greatest variety of those compounds, microalgae pose great potential as biotechnological producers.



Biomass productivity of common crops, versus microalgae (Dementer Bioscience 2023)

…with potential for production of terpenoids

Terpenoids comprise of a vast group of metabolites, present in all organisms. They have a wide range of biological functions, from pigments, vitamins, sex hormones and rubber to even serving as active pharmaceutical components, helping to fight cancer or malaria (Rodrigues & Lindberg, 2021). This makes them a valuable target for biotechnological production.

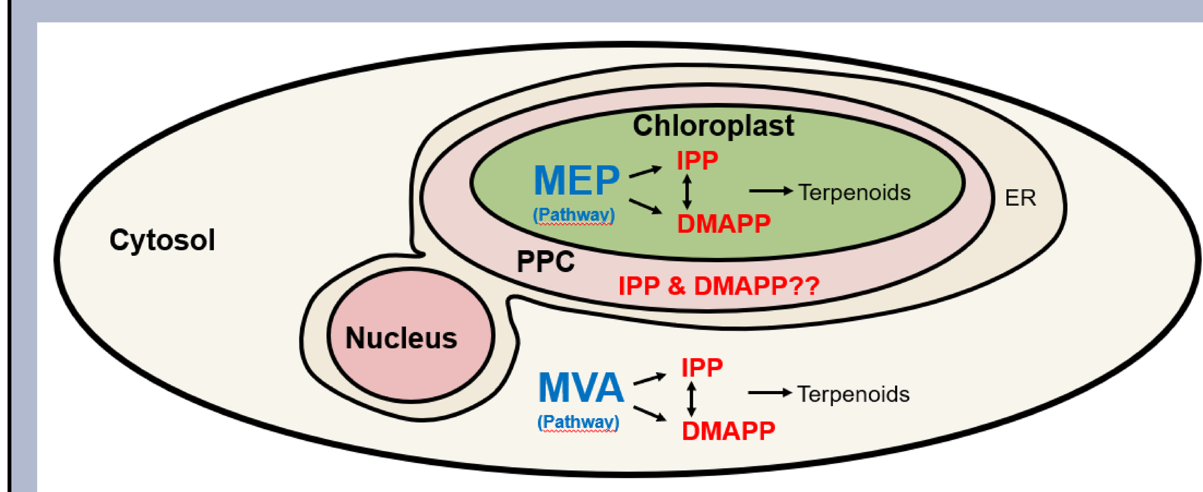


Different terpenoid classes with examples (Abdallah & Quax, 2017)

Terpenoid precursor synthesis in *P. tricornutum*

Terpenoids are synthesized in the cytosol and plastid of *P. tricornutum* by the mevalonate (MVA) and the Methyl-3-erythritol (MEP) pathway respectively. As terpenoid synthesis is tightly regulated (Y. Huang et al., 2021), it can be difficult to improve isoprenoid production. The diatom *P. tricornutum* carries an extra compartment – the periplastidal compartment (PPC) – which envelopes the chloroplast. Production pathways of high value terpenoids could be engineered to be located in this compartment, evading the tight regulation that might affect their productivity in the cytoplasm or the chloroplast, and ultimately enable higher production titers. Furthermore, concentrating the precursors in an extra, relatively small compartment will result in higher precursor concentrations and subsequently will increase the flux through succeeding enzymatic reactions. However first the questions remains to be answered, if the terpenoid precursors DMAPP and IPP are located in the PPC.

Experimental Outline - Are terpenoid precursors in the PPC?

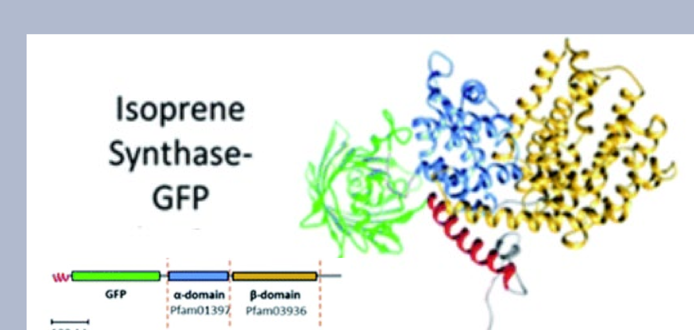


Shematic representation of P.tricornutum´s special compartmentilization and terpenoid metabolism

To answer the question whether terpenoid precursors are present in the PPC, a isoprene synthase (ISPS) of a poplar tree (Populus alba) is targeted by addition of signal peptides to the cytosol, the chloroplast and the PPC. ISPS take DMAPP to produce isoprene, which is a volatile compound that can be measured by gas chromatography (GC).

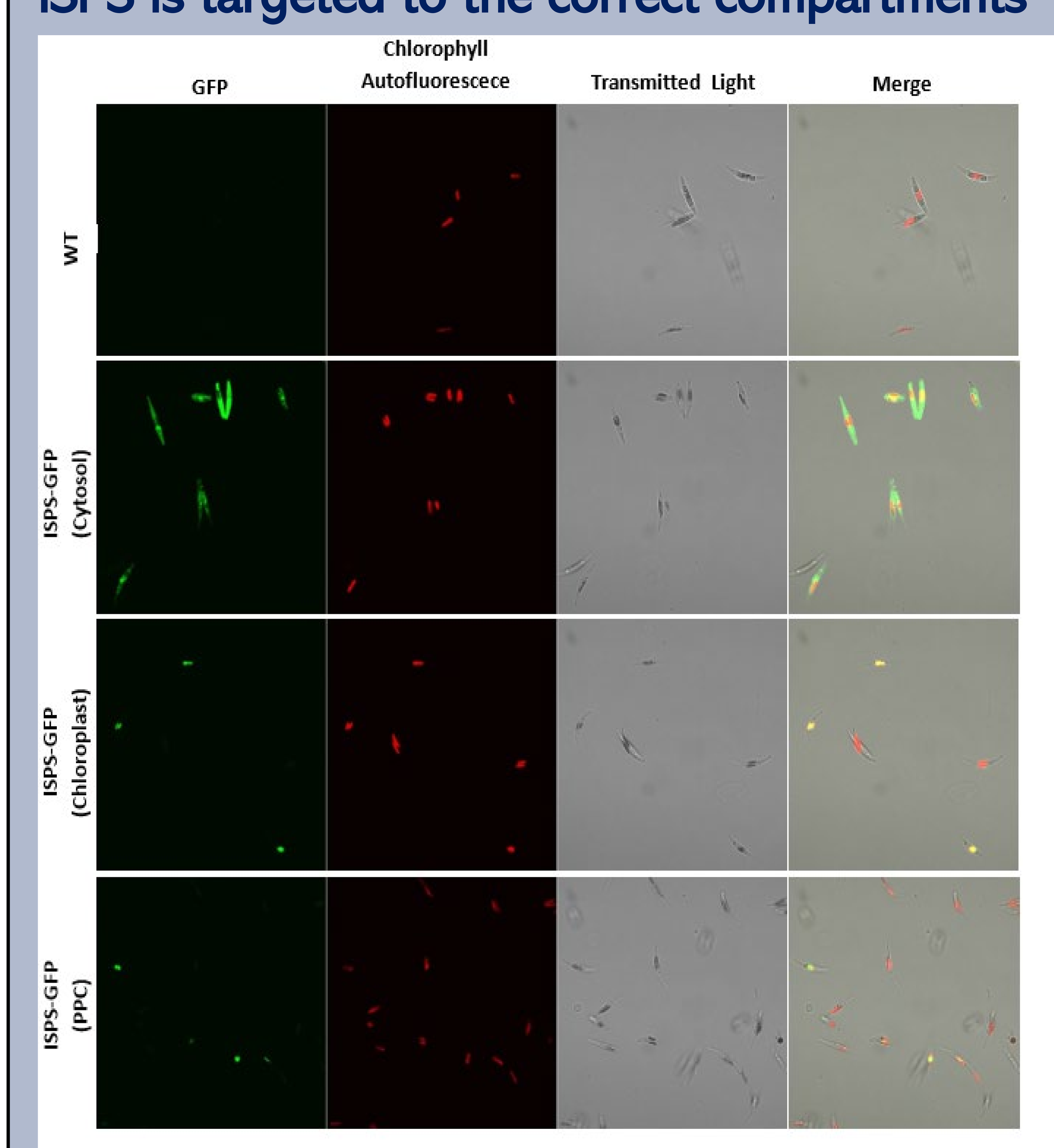
Questions to answer:

- Is ISPS targeted to the right compartments
- Quantification of isoprene production in different compartments
- How do the isoprene levels differ, when MEP or MVA specific inhibitors are added?



GFP-tagged ISPS (Gonzales-Esquer et al., 2021)

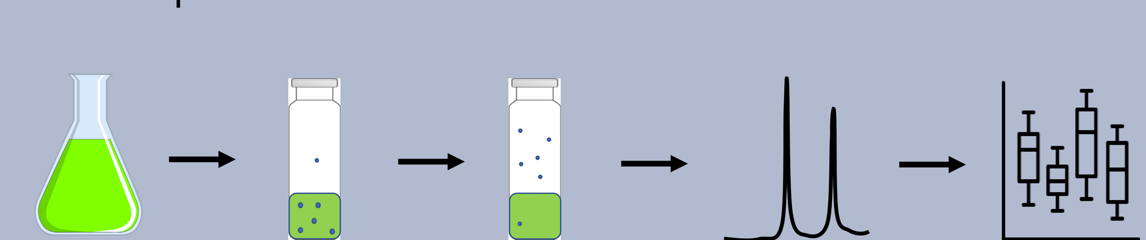
ISPS is targeted to the correct compartments



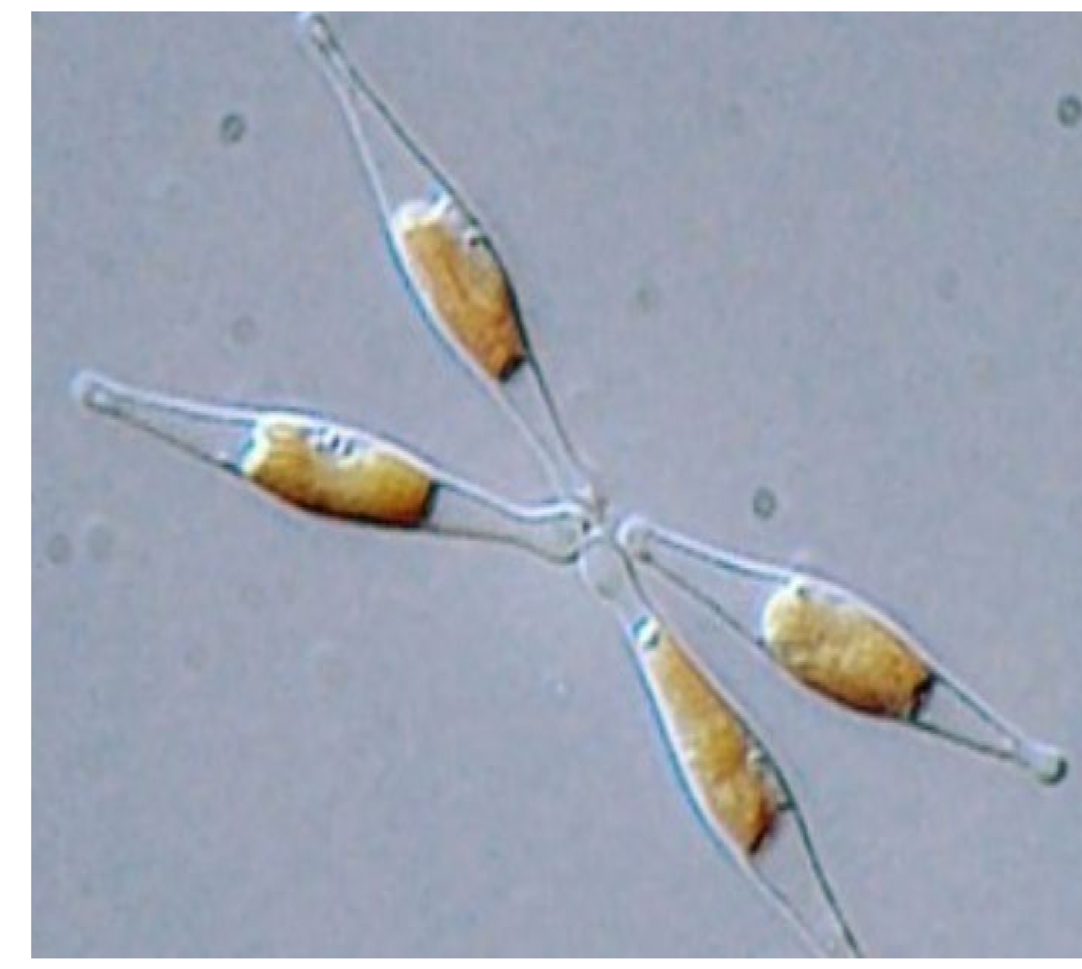
Confocal images of experimental lines and control.

Measurment of isoprene production

This will be the immediate next step in the project. The engineered algae lines are pre-cultured, concentrated and are grown in a gas-tight head-space (HS) vial. After three days of growth in the closed HS-vials, the cultrues are heated and the HS is analyzed via GC. The amount of produced isoprene will indicate whether the terpenoid precursor DMAPP is present in the PPC.



Shematic representation of the isoprene production and analysis part of the experiment.



Light microscopy acquisition of P. tricornutum (Alchetron 2023)

Outlook

As the experimental part of the project is still at an early phase, final results have not yet been obtained. The quantification of isoprene production of the obtained lines will be performed as indicated in the Methodology section. Furthermore, via inhibiting the MVA, the MEP or both pathways with specific inhibitors, light shall be shed on where from precursors (if any) are transported into the PPC.

If terpenoid pathway precursors are found in the PPC, it suggests their transport from the cytosol and/or plastid via transporters. Identifying these transporters would greatly enhance the PPC's potential as a terpenoid production site. Various approaches, such as overexpression library screening, CRISPRi library screens, or adaptive laboratory evolution experiments, can be employed to discover these transporters. These methods may also uncover transcription factors (TFs) associated with terpenoid synthesis activation. Utilizing the newly identified TFs and transporters through overexpression, a prototype algae can be developed for terpenoid production.

References

Araújo,R., Vázquez Calderón,F., Sánchez López,J., Azevedo,I. C., Bruhn, A., Fluch, S., Garcia Tasende, M., Ghaderiardakani,F., Ilmjärv,T., Laurans, M., mac Monagail,M., Mangini,S., Peteiro,C., Rebours,C., Stefansson, T., & Ullmann,J. (2021). Current Status of the Algae Production Industry in Europe: An Emerging Sector of the Blue Bioeconomy. Frontiersin MarineScience,7. https://doi.org/10.3389/fmars.2020.626389 Rodrigues,J. S., &

Lindberg,P. (2021). Engineering Cyanobacteria as Host Organisms for Production of Terpenes and Terpenoids. In Cyanobacteria Biotechnology (pp. 267–300). Wiley. https://doi.org/10.1002/9783527824908ch.9

Huang, Y., Xie, F.-J., Cao, X., & Li, M.-Y. (2021). Research progress in biosynthesis and regulation of plant terpenoids. Biotechnology & Biotechnological Equipment, 35(1), 1799–1808. https://doi.org/10.1080/13102818.2021.2020162

Demeter Biosciences. (2023). *Microalgal protein for food security*. https://www.demeterbioscience.com/microalgal-protein-for-food-security.html#/

Abdallah, I. I., & Quax, W. J. (2017). A Glimpse into the Biosynthesis of Terpenoids. KnE Life Sciences, 3(5), 81-98. https://doi.org/10.18502/kls.v3i5.981

Gonzalez-Esquer, C. R., Ferlez, B., Weraduwage, S. M., Kirst, H., Lantz, A. T., Turmo, A., Sharkey, T. D., & Kerfeld, C. A. (2021). Validation of an insertion-engineered isoprene synthase as a strategy to functionalize terpene synthases. RSC Advances, 11(48), 29997–30005. https://doi.org/10.1039/D1RA05710C

Alchetron: Phaeodactylum tricornutum. Alchetron.com. (2023. https://alchetron.com/Phaeodactylum-tricornutum