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Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable D2.1

Report on host strains with an increased pool and availability of photosynthetic intermediates as substrates for biofuels

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Editorial	
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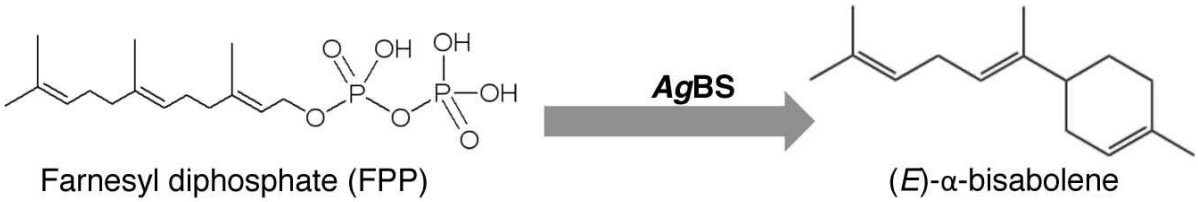
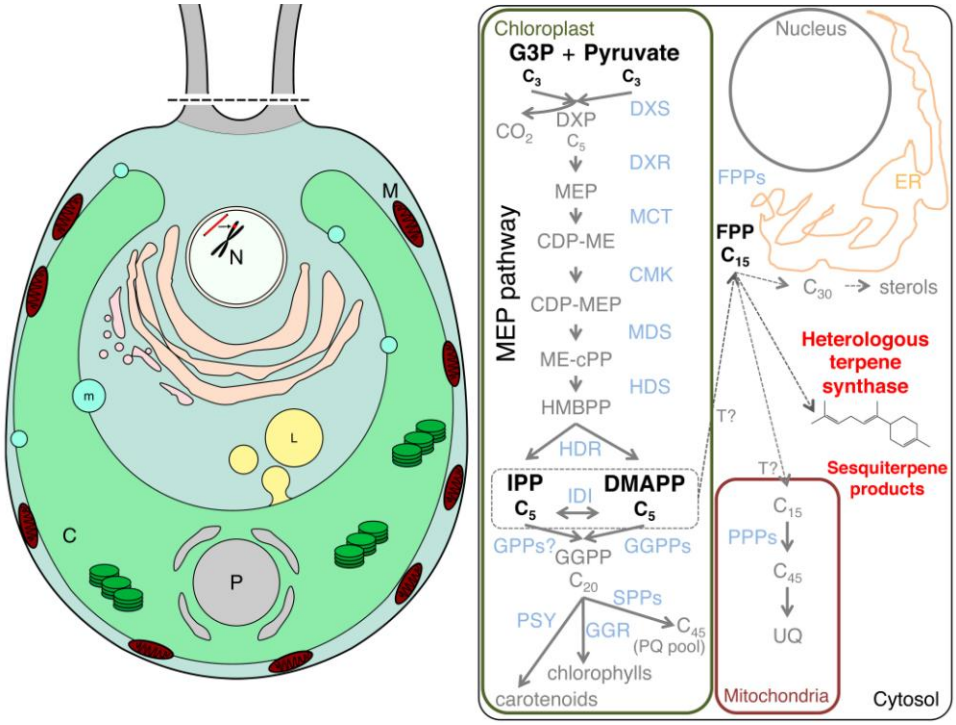
Publishable Summary

Sesquiterpenes, such as the recently characterized (*E*)-α-bisabolene can be extracted from the microbial cell without cell disruption and represent a unique class of products which may act as drop-in biodiesel replacements.



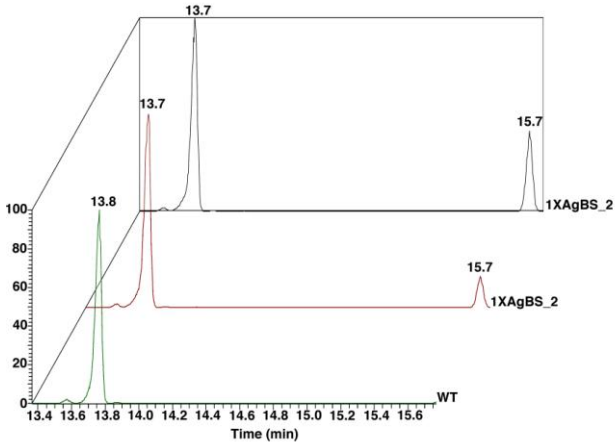
Conversion of farnesyl diphosphate to (*E*)-α-bisabolene by the *Abies grandis* bisabolene synthase (*Ag*BS).

Following recent developments in sesquiterpenoid production from the eukaryotic microalga *Chlamydomonas reinhardtii*, a codon optimized synthetic gene for the *Abies grandis* bisabolene synthase (*Ag*BS) was successfully transformed and expressed in this alga.



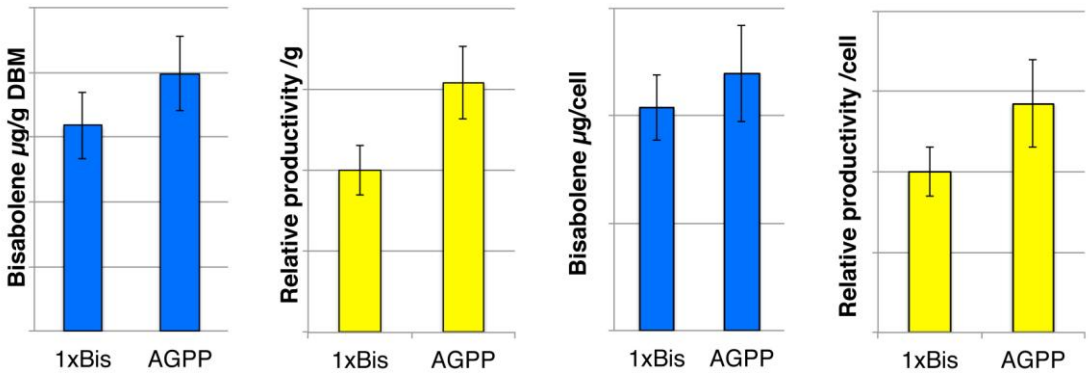
Cellular terpenoid metabolism in *C. reinhardtii*. Left, depiction of a *C. reinhardtii* cell, M mitochondria, N nucleus, m peroxisomal microbodies, L lipid droplets, C chloroplast, P pyrenoid (starch synthesis). To the right, the MEP pathway is depicted, all precursor enzymes except for the FPPs are found in the algal chloroplast. Through heterologous overexpression of a sesquiterpene synthase in the cytoplasm, the native FPP pool can be re-routed to form desired sesquiterpene products. Figure modified from: Lauersen, et al., 2016. Efficient phototrophic production of a high-value sesquiterpenoid from the eukaryotic microalga *Chlamydomonas reinhardtii*. Metabolic Engineering. 38. pp. 331-343.

Codon optimized and synthetic intron containing *Ag*BS bisabolene synthase – yellow fluorescent protein fusion from expression from the nuclear genome of *C. reinhardtii*.



Representative GS-MS chromatogram of dodecane overlays of two *Ag*BS expressing strains compared to the parental wild-type (WT). The internal standard α-humulene can be seen as a peak at ~13.7 min, while (*E*)-α-bisabolene is seen as a peak at ~15.7 min. Mass spectra (m/z) are set to 91.00, 93.00, 119.00.

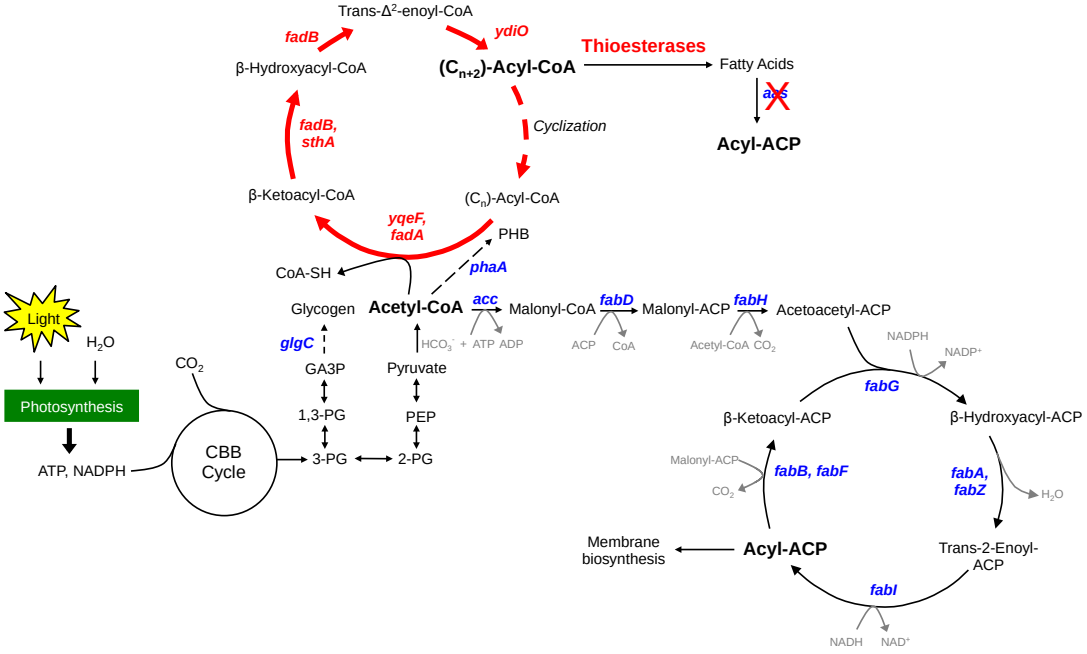
(*E*)-α-bisabolene was detected during two-phase cultivation with dodecane overlay as a sesquiterpene product produced from the algal cultures. This work has investigated the possibilities of improving yields by rational engineering of precursor enzyme fusions as well as knockdown of competitive pathways. Precursor fusions did not have a positive effect on (*E*)-α-bisabolene yields, while starch knockdown resulted in modest improvements in titres of up to ~1.57X (*E*)-α-bisabolene per dry biomass.



Knockdown of the AGPPs enzyme and subsequent reduction in starch accumulation has a positive effect on (*E*)-α-bisabolene production from *C. reinhardtii* grown in TAP media.

Further efforts will be focussed on other competitive pathway knockdowns, metabolic bypasses, and adaptive laboratory evolution to increase bisabolene titres from the algal host.

We also aim to produce fatty alcohols and alkanes in cyanobacteria. Towards this objective, we have implemented a synthetic pathway for fatty acid synthesis, introduced optimal parts and eliminated genes encoding enzymes that catalyze competing reactions, as summarized below. The analyses of the impact of these engineering efforts are still ongoing.



Proposed pathway of reverse β-oxidation cycle in engineered cyanobacteria. Blue: native enzymes; red: non-native enzymes; yqeF, fadA: thiolase; fadB: hydroxyacyl-CoA dehydrogenase; ydiO: enoyl-CoA reductase; sthA: transhydrogenase.