Dynamics of the Algal Plastid as a Vehicle for Sustainable Plant Terpene Production from Light and CO_2

Gordon B. Wellman, Malak Abdallah, Sebastian Overmans and Kyle J. Lauersen

Biological and Environmental Sciences and Engineering Division, KAUST, Saudi Arabia

جامعة الملك عبدالله للعلوم والتقنية
King Abdullah University of Science and Technology

Sustainable & Synthetic Biology Group (SSB)

C. reinhardtii as a Host

Chlamydomonas reinhardtii is a well-characterised eukaryotic unicellular alga from the *Viridiplantae* clade, distantly related to land plants. It possesses a single chloroplast and is capable of photosynthetic growth using light and CO_2, as well as mixotrophic and heterotrophic growth when supplied with a carbon source in the form of acetate. Recent strain development and synthetic biology facilitated transgene design has overcome gene silencing allowing for heterologous enzyme expression in a 'plant-like' environment with the possibility of being able to be grown like other microbes. For these reasons as well as its generally regarded as safe (GRAS) status makes *C. reinhardtii* an exciting cell chassis for heterologous production of various high-value terpenes, such as the sesquiterpenoid patchoulol, as well as the keto-carotenoid, astaxanthin.

Terpene Biosynthesis

C. reinhardtii relies solely on the methyl-D-erythritol phosphate (MEP) pathway localized in its plastid for isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) production. These are exported to the cytosol for conversion to 15-carbon (2*E*,6*E*)-farnesyl diphosphate (FPP) for squalene biosynthesis or used within the chloroplast for carotenoid biosynthesis. Expression of *Pogostemon cablin* patchoulol synthase (*Pc*PS) allows conversion of free FPP into patchoulol in both cytosol and plastid when appropriately targeted.

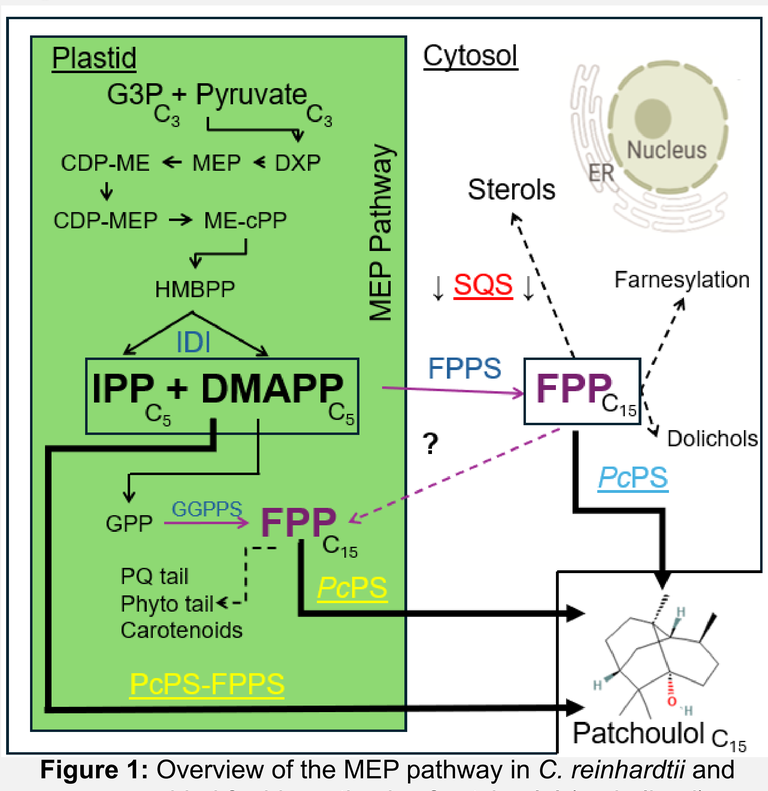

Figure 1: Overview of the MEP pathway in *C. reinhardtii* and genes added for biosynthesis of patchoulol (underlined)

Engineering for Patchoulol Production

A lab-developed strain of *C. reinhardtii* was transformed step-wise with fluorescently tagged patchoulol synthases (*Pc*PS) targeted to either the cytosol and/or plastid (**Fig.2**). To improve patchoulol titres we also modified the FPP pools in the sub-cellular compartments by knock-down of the squalene synthase (SQS-k.d) and expression of plastid targeted FPP synthase (FPPS) (**Fig.1**)

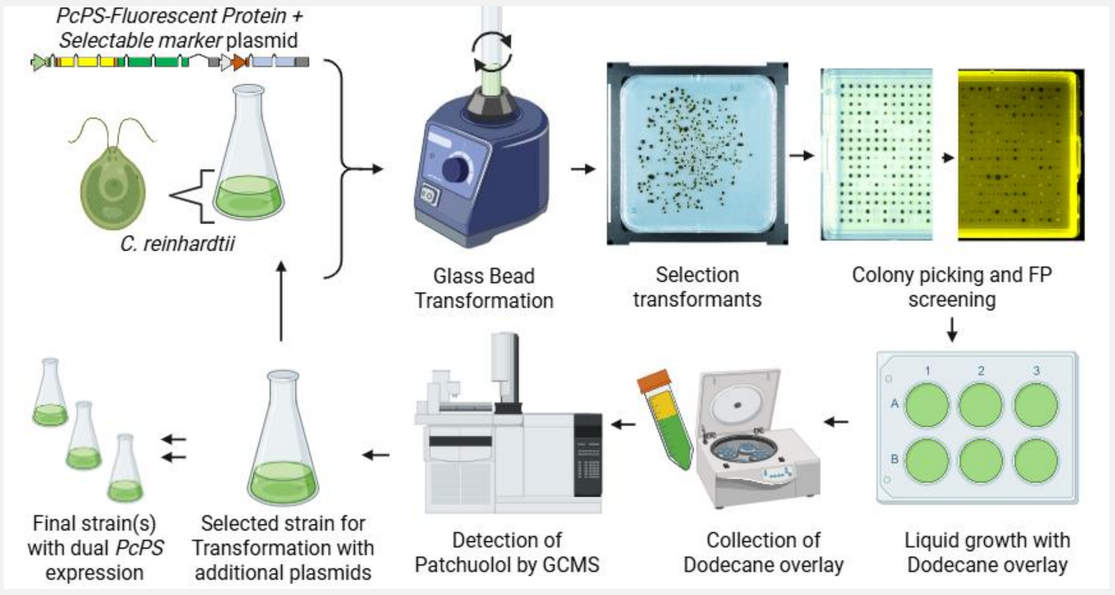

Figure 2: Transformation and selection of *C. reinhardtii* and screening for patchoulol by GCMS

Productivity Changes Under Different Growth Conditions

Selected strains with dual cytosol or plastid targeted *Pc*Ps, SQS-k.d and/or plastid FPPS with similar *Pc*Ps expression levels determined by SDS-PAGE were selected and grown in 400 mL Algem photobioreactors (**Figure 3**). Strains were subjected to different light regimes and carbon sources (3% CO_2,~1g/L acetate or both) using a perfluoro-solvent underlay (3M™ Fluorinert™ FC-40) to capture patchoulol. Daily productivity was measured by flow cytometry and detection of patchoulol in the underlay by Gas Chromatography-Mass Spectrometry (GCMS)

Figure 3: Algem Photobioreactors used for light and carbon source growth trials

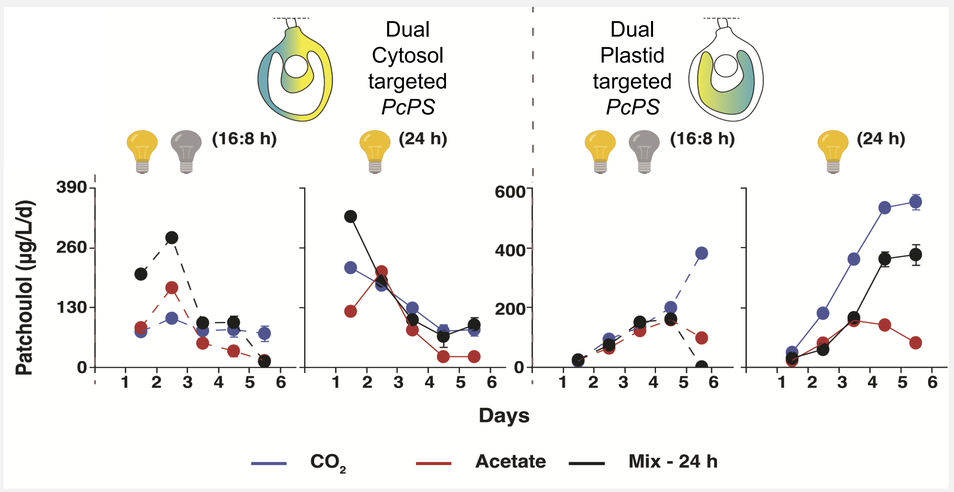

Figure 4: Daily productivity of dual *Pc*PS expressing strains grown in Algem

The dual *Pc*PS expressing strains demonstrated different productivity dynamics over the course of the 6-day experiment based on the carbon source and cytosol vs. plastid targeting (**Figure 4**) while yielding similar amounts by the end of the experiment. The reduction in productivity in acetate-only grown cultures is likely a result of limited carbon source.

High Density Cultivation Under Photosynthetic conditions

To assess CO_2-based high-density growth, selected strains above were further tested in CellDEG HD100 (100mL) photobioreactors using nutrient enhanced acetate-free medium (6xPhi) and 5% CO_2 as the sole carbon source. This system uses a thin culture layer and gas-exchange membrane to enhance phototrophic growth to high densities. A dodecane overlay was used to capture patchoulol. Daily cell counts by flow cytometry and patchoulol was measured by GCMS.

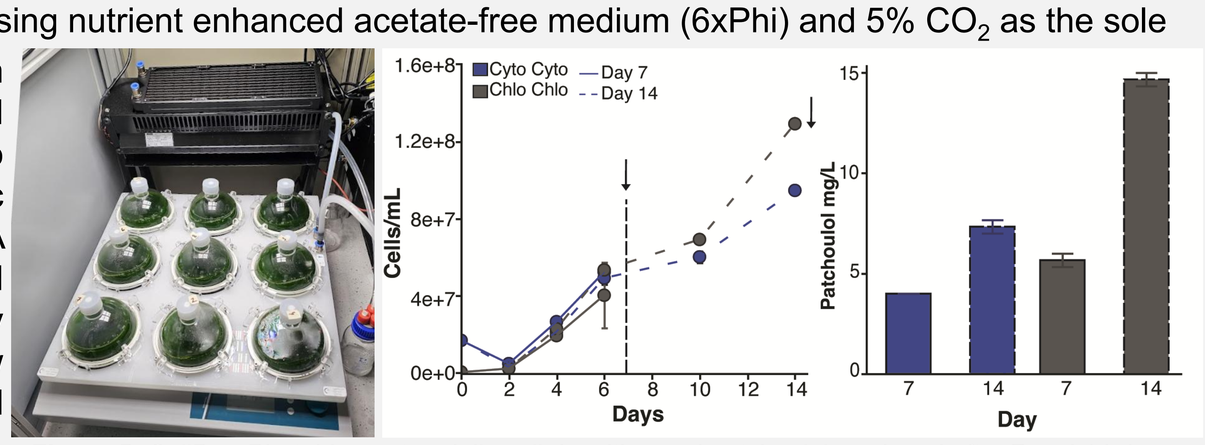

Figure 5: CellDeg Photobioreactor, daily cell densities and patchoulol produced after 7 and 14 days

More Information

Digital Version

Contact:

@GordonWellman

Gordon.Wellman@KAUST.edu.sa

Current Pre-Print

Conclusions & Future Work

Targeting of *Pc*PS to the cytosol led to higher productivity under mixotrophic in early growth stages, while targeting to the plastid led to higher productivity under photosynthetic growth in later growth stages. In high density growth trials, we were able to produce ~14 mg/L of culture patchoulol. These insights from the basis of further work in both engineering steps and bioreactor design and feeding strategies to maximise production of patchoulol and other valuable terpenoids using *C. reinhardtii* as a green synthetic host. Further modification of the MEP pathway may increase the flow of carbon to desired heterologous terpenoids.