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### **ACCEPTED MANUSCRIPT**

# Efficient phototrophic production of a high-value sesquiterpenoid from the eukaryotic microalga Chlamydomonas reinhardtii

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### Abstract

The heterologous expression of terpene synthases in microbial hosts has opened numerous possibilities for bioproduction of desirable metabolites. Photosynthetic microbial hosts present a sustainable alternative to traditional fermentative systems, using freely available (sun)light and carbon dioxide as inputs for bio-production. Here, we report the expression of a patchoulol synthase from Pogostemon cablin Benth in the model green microalga Chlamydomonas reinhardtii. The sesquiterpenoid patchoulol was produced from the alga and was used as a marker of sesquiterpenoid production capacity. A novel strategy for gene loading was employed and patchoulol was produced up to  $922 \pm 242 \ \mu g \ g^{-1} \ CDW$  in six days. We additionally investigated the effect of carbon source on sesquiterpenoid productivity from C. reinhardtii in scale-up batch cultivations. It was determined that up to  $1.03 \ m g \ L^{-1}$  sesquiterpenoid products could be produced in completely photoautotrophic conditions and that the alga exhibited altered sesquiterpenoid production metabolism related to carbon source.

**Keywords:** Microalgae. Chlamydomonas reinhardtii. Terpenoids. Sesquiterpenoids. Patchoulol.

#### Abbreviations

- PcPs Pogostemon cablin Benth patchoulol synthase
- TAP Tris Acetate Phosphate medium

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