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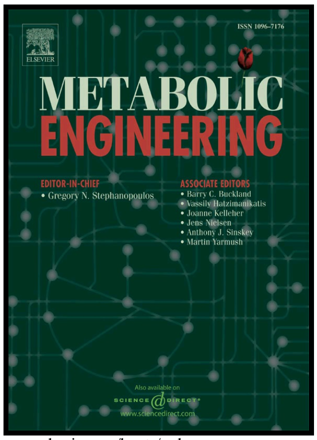

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Synthetic metabolic pathways for photobiological conversion of CO_2 into hydrocarbon fuel

Ian Sofian Yunus[a], Julian Wichmann[b], Robin Wördenweber[b], Kyle J. Lauersen[b], Olaf Kruse[b], Patrik R. Jones[a*]

[a]Department of Life Sciences, Imperial College London, SW7 2AZ London, UK
[b]Bielefeld University, Faculty of Biology, Center for Biotechnology (CeBiTec), Universitätsstrasse 27, 33615, Bielefeld, Germany.

*Corresponding author. p.jones@imperial.ac.uk

ABSTRACT

Liquid fuels sourced from fossil sources are the dominant energy form for mobile transport today. The consumption of fossil fuels is still increasing, resulting in a continued search for more sustainable methods to renew our supply of liquid fuel. Photosynthetic microorganisms naturally accumulate hydrocarbons that could serve as a replacement for fossil fuel, however productivities remain low. We report successful introduction of five synthetic metabolic pathways in two green cell factories, prokaryotic cyanobacteria and eukaryotic algae. Heterologous thioesterase expression enabled high-yield conversion of native fatty acyl-acyl carrier protein (ACP) into free fatty acids (FFA) in *Synechocystis sp.* PCC 6803 but not in *Chlamydomonas reinhardtii* where the polar lipid fraction instead was enhanced. Despite no increase in measurable FFA in *Chlamydomonas*, genetic recoding and over-production of the native fatty acid photodecarboxylase (FAP) resulted in increased accumulation of 7-heptadecene. Implementation of a carboxylic acid reductase (CAR) and aldehyde deformylating oxygenase (ADO) dependent synthetic pathway in *Synechocystis* resulted in the accumulation of fatty alcohols and a decrease in the native saturated alkanes. In contrast, the replacement of CAR and ADO with *Pseudomonas mendocina* UndB (so named as it is responsible for 1-undecene biosynthesis in *Pseudomonas*) or *Chlorella variabilis* FAP resulted in high-yield conversion of thioesterase-liberated FFAs into corresponding alkenes and alkanes, respectively. At best, the engineering resulted in an increase in hydrocarbon accumulation of 8- (from 1 to 8.5 mg/g cell dry weight) and 19-fold (from 4 to 77 mg/g cell dry weight) for *Chlamydomonas* and *Synechocystis*, respectively. In conclusion, reconstitution of the eukaryotic algae pathway in the prokaryotic cyanobacteria host generated the most