ABSTRACT

Cyanobacteria are photosynthetic prokaryotes and important primary producers which can directly convert atmospheric CO_2 into economically viable biochemicals using solar energy. Owing to their efficient photosynthesis ability than other photoautotrophs including plants, simple prokaryotic cell organization and natural competency to uptake extracellular DNA; cyanobacteria have been widely explored as cyanofactories for production of value-added compounds and hydrocarbons. Engineering these cells for chemical synthesis has been potential green bioconversion approach, whereby more than 20 chemicals have been successfully produced by engineered cyanobacteria. In light of this perspective, we have exploited cyanobacteria *Synechococcus elongatus* PCC 7942 (fresh water strain) and *Synechococcus sp.* PCC 7002 (marine strain) for both; improving biomass productivities as well as hydrocarbon production under natural light conditions. Therefore, the work has been broadly categorized into two sections; cyanobacterial growth studies and cyanobacterial genetic engineering for hydrocarbon production.

Preliminary light acclimation studies have been performed to comprehend the effect of natural light on cyanobacterial growth and photosynthetic pigment profiles. These studies showed that cyanobacteria could efficiently grow under natural diurnal light conditions. This improved growth rate is concurrent with reduced photo-toxicity by decreasing photosynthetic antenna density. This pigment modulation ability helps the cells in faster acclimatization to dynamic light alterations. Therefore, further investigations were performed under sunlight. To increase biomass productivity, we examined ability of various cyanobacteria to uptake extracellular organic C. PCC 7942 and PCC 7002 which cannot naturally uptake extracellular sugar, were engineered to display heterologous sugar transporters and perform mixotrophy. Integrative plasmid for PCC 7942 was commercially available, however we constructed an indigenous plasmid vector *pUKD6* for genetic engineering of PCC 7002 cells and could successfully demonstrate mixotrophy in the strain.

Efforts have been made to genetically engineer PCC 7942 cells for heterologous production of isoprene where we could produce 14 µg/L.h bioisoprene. Moreover, PCC 7942 cells were also modified for improving the flux towards value-added xanthophyll zeaxanthin, where our engineered cells exhibited 2.5 times improved yield over wild type culture. Simultaneously, we engineered these cells for mixotrophy as already explained above. Conjoining these strategies was found to increase the yield of lutein-free zeaxanthin while improving cyanobacterial biomass productivity, thereby escalating the product titer thrice over wild type. In addition, we explored PCC 7942 chassis for production of novel

biopharmaceutical molecule heparosan through metabolic engineering. Our studies demonstrated for the first-time engineered cyanobacteria for the photoautotrophic production of this high-value polysaccharide.

Collectively, our work provides insights to cyanobacterial growth and genetic engineering for production of hydrocarbons under natural light conditions.

Keywords: Algae, Cyanobacteria, Hydrocarbons, Carotenoids, Genetic engineering, Value-added biochemicals, Photochemistry

Classification: Biotechnology, Molecular Biology, Algal Biotechnology, Cyanobacteria Genetic Engineering

Abbreviations:

Auto, Autotrophy

EC/EL, Environmental chamber/ laboratory (State of the art glass house facility at DBT-ICT Centre for Energy Biosciences)

IC, Incubator

Tr, transformant

WT, Wild Type