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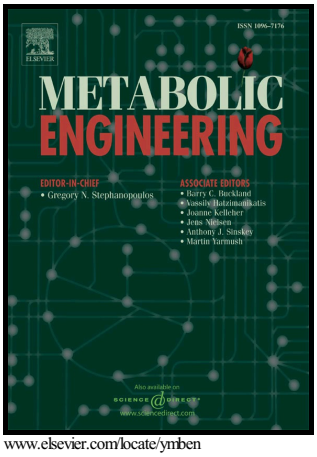

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Engineered cyanobacteria with enhanced growth show increased ethanol production and higher biofuel to biomass ratio

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Abstract

The Calvin-Benson-Bassham (CBB) cycle is the main pathway to fix atmospheric CO_2 and store energy in carbon bonds, forming the precursors of most primary and secondary metabolites necessary for life. Speeding up the CBB cycle theoretically has positive effects on the subsequent growth and/or the end metabolite(s) production. Four CBB cycle enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), fructose-1,6/sedoheptulose-1,7-bisphosphatase (FBP/SBPase), transketolase (TK) and aldolase (FBA) were selected to be co-overexpressed with the ethanol synthesis enzymes pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) in the cyanobacterium *Synechocystis* PCC 6803. An inducible promoter, P*nrsB*, was used to drive PDC and ADH expression. When P*nrsB* was induced and cells were cultivated at 65μmol photons m^{-2} s^{-1}, the RuBisCO-, FBP/SBPase-, TK-, and FBA-expressing strains produced 55%, 67%, 37% and 69% more ethanol and 7.7%, 15.1%, 8.8% and 10.1% more total biomass (the sum of dry cell weight and ethanol), respectively, compared to the strain only expressing the ethanol biosynthesis pathway. The ethanol to total biomass ratio was also increased in CBB cycle enzymes expressing strains. This study experimentally demonstrates that using the cells with enhanced carbon fixation, when the product synthesis pathway is not the main bottleneck, can significantly increase the generation of a product (exemplified with ethanol), which acts as a carbon sink.

Keywords

Cyanobacteria, Biofuel, Carbon fixation, Ethanol, RuBisCO, FBP/SBPase, TK, FBA

1. Introduction