Design, construction and introduction of artificial DNA encoding a FeFe hydrogenase into unicellular cyanobacterial cells

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H_2 , one of the immaculate fuel available till date which accounts for almost zero emission acts as the major potential renewable biofuel that can come into play by helping to switch off the global warming. In our case we are interested to produce H_2 gas biologically using cyanobacterial biomass with the use of sunlight as the energy source and hence called bio-hydrogen production. This phototrophic organism can produce biohydrogen via photosynthetic reaction. During photosynthesis the water molecule is broken into protons, electrons and O_2 molecules. The liberated protons are then reduced with the help of hydrogenase enzyme to produce molecular H_2.

Photosynthesis reaction: $2H_2O \rightarrow 4H^+ + 4e^- + O_2$

Hydrogen production reaction: $4H^+ + 4e^- \rightarrow 2H_2 + 2e^-$

The enzyme hydrogenase plays an important role in catalysis of above H_2 production reaction. In our case the cyanobacterial strain that we use is *Synechocystis sp.* PCC 6803, has a native [NiFe]-hydrogenase. The researches show that [FeFe]-hydrogenase which is only present in some eukaryotice microbes and prokaryotes are much efficient in H_2 production when expressed heterologously in *Synechocystis sp.* PCC 6803. Our attempt here is to engineer this cyanobacteria to express highly efficient [FeFe]-hydrogenase encoded by functional gene hydA by replacing its native [NiFe]-hydrogenase. For this to accompany the project focuses on making an artificial DNA that contains functional gene hydA from *Clostridium acetobuatylicum* encoding for [FeFe]-hydrogenase along with its maturation genes hydE, hydF and hydG for its proper folding and maturation. This construct will be then finally integrated into the genome of *Synechocystis sp.* PCC 6803 via homologous recombination.

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