DESIGN, CONSTRUCTION AND INTRODUCTION OF ARTIFICIAL DNA ENCODING ALGAL HYDROGENASE INTO FILAMENTOUS CYANOBACTERIA

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The rise in petrol prices and adverse effect of greenhouse gas as well as concern regarding the oil consumption has raised interest in the search for alternative fuels. Many researchers are interested in using Hydrogen as alternative fuel but the problem lies in production of hydrogen fuel at a large scale. Metabolic engineering was suggested as a good strategy since it could be used for eliminating bottlenecks, increase carbon flow to hydrogen produce pathway and engineer more efficient oxygen resistant hydrogenases.The use of hydrogenase enzymes for hydrogen production has been more of interest as it can be used for microbial production of biohydrogen.The microbial production of biohydrogen is dependent on the H_2 metabolism mechanism which is very efficient.The hydrogenase enzymes which are responsible for this mechanism catalyzes the following reaction:

$2H^+ + 2e^- \Leftrightarrow H_2$

For the above redox reaction to take place special maturation proteins and metalloclusters as active sites are required. The aim of the project was to express green algal hydrogenase in a cyanobacterial strain by designing a suitable genetic construct.In this project,more emphasis was given on the use of [FeFe]-hydrogenases for increasing the production of hydrogen in filamentous cyanobacterial strain since they are highly efficient,due to their high activity and specificity.[FeFe]-hydrogenase encoded by functional gene hydA was used for replacing its native [NiFe]-hydrogenase. The project focused 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 would be then finally integrated into the genome of cyanobacterium Anabaena sp strain,7120bp, through homologous recombination. hydrogenase.Initially Gibson assembly method was selected the formation of construct as it was considered to be less time consuming and efficient method for formation of constructs.It was also decided to use over lap extension PCR if the Gibson assembly did not give satisfactory results.The over lap extension PCR was selected as another option due its high specificity,which might decrease the chances of getting false results,even though it has a disadvantage of being time consuming.

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