

## Increasing Photosynthetic H<sub>2</sub> Production by in vivo Expression of Re-Engineered Ferredoxin-Hydrogenase Fusion Protein in the Green Alga *Chlamydomonas reinhardtii*

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**Abstract :** The most urgent challenge of our time is to replace the depleting resources of fossil fuels by sustainable environmentally friendly alternatives. Hydrogen is a promising CO<sub>2</sub>-neutral fuel for a more sustainable future especially when produced photo-biologically. Hydrogen can be photosynthetically produced in unicellular green alga like *Chlamydomonas reinhardtii*, catalysed by the inducible highly active and bidirectional [FeFe]-hydrogenase enzymes (HydA). However, evolutionary and physiological constraints severely restrict the hydrogen yield of algae for industrial scale-up, mainly due to its competition among other metabolic pathways on photosynthetic electrons. Among them, a major challenge to be resolved is the inferior competitiveness of hydrogen production (catalysed by HydA) with NADPH production (catalysed by ferredoxin-NADP<sup>+</sup>-reductase (FNR)), which is essential for cell growth and takes up ~95% of photosynthetic electrons. In this work, the in vivo hydrogen production efficiency of mutants with ferredoxin-hydrogenase (Fd\*-HydA1\*) fusion protein construct, where the electron donor ferredoxin (Fd\*) is fused to HydA1\* and expressed in the model organism *C. reinhardtii* was investigated. Once Fd\*-HydA1\* fusion gene is expressed in algal cells, the fusion enzyme is able to draw the redistributed photosynthetic electrons and use them for efficient hydrogen production. From preliminary data, mutants with Fd\*-HydA1\* transgene showed a ~2-fold increase in the photosynthetic hydrogen production rate compared with its parental strain, which only possesses the native HydA in vivo. Therefore, a solid method of having more efficient hydrogen production in microalgae can be achieved through the expression of the synthetic enzymes.

**Keywords :** *Chlamydomonas reinhardtii*, ferredoxin, fusion protein, hydrogen production, hydrogenase

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