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Redirecting the electron flow towards the nitrogenase and bidirectional Hox-hydrogenase by using specific inhibitors results in enhanced H_2 production in the cyanobacterium *Anabaena siamensis* TISTR 8012

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HIGHLIGHTS

- Photosynthetic cyanobacterial based H_2 production is limited by electron supply.
- Redirecting competing pathways for electron flow results in increased H_2 production.
- Metabolic engineering leads to increased production of desired product.

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ABSTRACT

The inhibition of competitive metabolic pathways by various inhibitors in order to redirect electron flow towards nitrogenase and bidirectional Hox-hydrogenase was investigated in *Anabaena siamensis* TISTR 8012. Cells grown in $BG11_0$ supplemented with KCN, rotenone, DCMU, and DL-glyceraldehyde under light condition for 24 h showed enhanced H_2 production. Cells grown in BG11 medium showed only marginal H_2 production and its production was hardly increased by the inhibitors tested. H_2 production with either 20 mM KCN or 50 μM DCMU in $BG11_0$ medium was 22 μmol H_2 mg chl a^{-1} h^{-1}, threefold higher than the control. The increased H_2 production caused by inhibitors was consistent with the increase in the respective Hox-hydrogenase activities and *nifD* transcript levels, as well as the decrease in *hupL* transcript levels. The results suggested that interruption of metabolic pathways essential for growth could redirect electrons flow towards nitrogenase and bidirectional Hox-hydrogenase resulting in increased H_2 production.

1. Introduction

H_2 metabolism in N_2-fixing cyanobacteria may involve three enzymes: nitrogenase, the uptake hydrogenase and the bidirectional Hox-hydrogenase. The nitrogenase complex consists of two complexes, dinitrogenase (encoded by *nifD* and *nifK*) and dinitrogenase reductase (encoded by *nifH*). Nitrogenase catalyzes H_2 production concomitantly with N_2-fixation with a minimum of 25% of the electrons used for H_2 production. The uptake hydrogenase consists of at least two subunits (encoded by *hupS* and *hupL*) oxidizing the H_2 evolved by nitrogenase. The bidirectional Hox-hydrogenase consists of a hydrogenase part (encoded by *hoxY* and *hoxH*) and a diaphorase part (encoded by *hoxE*, *hoxU* and *hoxF*). The enzyme may catalyze both the production and utilization of H_2 (Tamagnini et al., 2007; Phunpruch et al., 2006). N_2-fixing cyanobacteria are suitable for photobiological hydrogen production. They can use solar energy to split water and shuttle electrons through the electron transport chain to the terminal electron acceptor ferredoxin (Fd) via the plastoquinone pool. This will generate strong reductants such as NADPH and reduced ferredoxin, which can be utilized as substrates for H_2 production by either nitrogenase or hydrogenase (Lubitz et al., 2008 and Tamagnini et al., 2007) (Fig. 1). However, major obstacles for a sustainable H_2 production in N_2-fixing cyanobacteria are the irreversible inhibition of the enzymes by oxygen, H_2 consumption by the uptake hydrogenase and an overall low H_2 productivity due to the competition for electrons by numerous other assimilatory pathways. The

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