Overproduction of the Flv3B flavodiiron, enhances the photobiological hydrogen production by the nitrogen-fixing cyanobacterium *Nostoc* PCC 7120

CURRENT STATUS: ACCEPTED

Microbial Cell Factories BMC

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DOI:

10.21203/rs.2.19563/v1

SUBJECT AREAS

Applied & Industrial Microbiology

KEYWORDS

Cyanobacteria, Flavodiiron, Heterocyst, Hydrogen, Hydrogenase

Abstract

Background: The ability of some photosynthetic microorganisms, particularly cyanobacteria and microalgae, to produce hydrogen (H_2) is a promising alternative for renewable, clean-energy production. However, studies of the topic in the last decade have shown that much improvement is needed before sustainable cyanobacterial-based H_2 production becomes economically viable. In this study, we investigated the impact of inducing O_2 -consumption to enhance H_2 photoproduction yields in the heterocyst-forming, N_2 -fixing cyanobacterium *Nostoc* PCC7120.

Results: The *flv3B* gene, encoding a flavodiiron protein naturally expressed in the heterocyst of *Nostoc*, was overexpressed. Compared to the wild type, the recombinant strain obtained displayed a significantly higher H_2 production under aerobic growth and phototrophic conditions. Nitrogenase activity assays indicated that *flv3B* overexpression did not increase the nitrogen fixation rates. On the other hand, quantitative RT-PCR experiments showed that the transcription of the *hox* genes, encoding the NiFe Hox hydrogenase was greatly elevated in the *flv3B* overexpressing strain.

Conclusion: We conclude that the overproduced Flv3B protein might have enhanced O_2 -consumption, thus creating conditions inducing *hox* genes and facilitating H_2 production. The present study clearly demonstrates the potential to use metabolic engineered cyanobacteria for photosynthesis driven H_2 production.

Full Text

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However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Flv3B overproduction analysis A, B, C: Quantitative RT-PCR analysis of *flv3B* and *patB* gene transcription. RNA were collected from the wild type or the WT/*patB-flv3B* strain at four different times (7, 18, 24 and 48 hours) after the onset of nitrogen depletion. Each sample was measured in triplicate and the standard deviation is indicated by error bars. Values were normalized to the *rnpB* transcript (A/B/C), relatively to the value obtained for the wild type strain, which was set to 1. D: Immunoblot analysis of the amount of Flv3B protein in the wild type and WT/*patB-flv3B* strains, carried out using antibodies produced against FlvB from *Chlamydomonas reinhardtii* [18]. Immunoanalysis of RbcL protein amount was carried out as a loading control. BG11: nitrate-containing medium; BG110: nitrate free medium.

Figure 2

Characterization of Nostoc strain overexpressing the *flv3B* gene A: Growth curve of Nostoc strains grown in either BG11 (nitrate-containing medium) or BG110 (nitrate free medium). B: Light microscope images of Nostoc strains grown in either BG11 or BG110. Heterocysts are indicated by arrows.

Figure 3

H₂ production kinetics Time zero indicates the onset of the nitrogen depletion. Means $\pm$ SEM (n=8).

Figure 4

hoxY, *H* genes transcription analysis Quantitative RT-PCR analysis of *hoxY* and *hoxH* gene transcription. RNA were collected from wild type (A, B) or WT/*patB-flv3B* (C, D) at different times after the onset of the nitrogen depletion step. Each sample was measured in triplicate and the standard deviation is indicated by error bars. Values were normalized to the *rnpB* transcript.

Figure 5

hoxE, *F* genes transcription analysis. Quantitative RT-PCR analysis of *hoxE* and *hoxF* gene transcription. RNA were collected from wild type (A, B) or WT/*patB-flv3B* (C, D) at different times after the onset of the nitrogen depletion step. Each sample was measured in triplicate and the standard deviation is indicated by error bars. Values were normalized to the *rnpB* transcript.