**Light-induced promoter drives efficient over-expression of alternative nitrogenases in genetically modified *Rhodopseudomonas palustris***

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**Highlights**

* Volumetric biohydrogen production rate is limited by poor Mo nitrogenase enzyme activity
* V and Fe nitrogenase over-expression by insertion of efficient promoters to override control
* *PucBa* promoter shows promise for high-level, light-induced heterologous gene expression
* Potential of *R. palustris* as photobiological chassis organism is further advanced.

**1. Introduction**

Biohydrogen has long shown great promise as a clean energy source, produced from organic wastes by metabolically versatile anoxygenic photosynthetic bacteria such as *Rhodopseudomonas palustris*. Despite decades of investigation, feasibility of this potential bio-economy technology at large-scale remains limited by low volumetric hydrogen production rates [1].

The molybdenum (Mo) nitrogenase enzyme, the activity of which yields hydrogen as an obligate by-product, is driven by energy in the form of ATP derived from photosynthesis and electrons from organic substrates according to:

N2 + 8H+ + 8e- + 16 ATP → 2NH3 + H2 + 16 ADP

This complex, energetically expensive reaction results in a slow catalytic turnover, likely resulting in a bottleneck in the hydrogen production pathway [2]. Previous work on the temperature dependence of H2 production supports this hypothesis: an increase in production rate was observed up to 40°C under equivalent light intensity, indicating availability of surplus reducing power to drive the reaction under thermodynamic conditions favouring higher enzyme catalysis rates. The energetic cost of nitrogenase activity means expression is tightly-repressed by availability of NH3, limiting the potential for hydrogen production from organic waste waters containing a ubiquitous nitrogenous substrate [3].

*R. palustris* possesses two alternative nitrogenases with iron (Fe) and vanadium (V) cofactors, each evolving 3 and 9 H2 per N2 reduced respectively [4]. A prime target for addressing poor nitrogenase activity is thus presented, in overexpressing these isozymes via genetic modification.

Here we present the genetic modification of *R. palustris* by insertion of efficient promoters upstream of native nitrogenase gene operons in order to circumvent innate control mechanisms, and the characterisation of gene expression in the resultant strains.

**2. Methods**

*Rhodopseudomonas palustris* putative promoter sequences from LH2 protein (*pucBa*: light & anaerobic induced) or citrate synthase (*cisY*: constitutive expression) were inserted upstream of all 3 native nitrogenase gene operons by homologous recombination methods. Nitrogenase gene expression in the six resultant unmarked strains was quantified by RT-qPCR.

**3. Results and discussion**

Six strains of nitrogenase-overexpressing R. palustris were generated using an optimised protocol. Detailed quantification of transcript levels by RT-qPCR showed efficient upregulation of all genes in nitrogenase operons driven by puc promoter, between 8 to 12 Ct values higher than wild-type (up to ~4000-fold overexpression). Strains under control of cit promoter showed variable success with good upregulation of Vnf but not Anf, suggesting locus-dependent activity.



**Figure 1.** Quantification of gene expression from *R. palustris* vanadium (Vnf) and iron (Anf) nitrogenase operons

**4. Conclusions**

We present an efficient method for generating strains of *R. palustris* overexpressing complex native, multi subunit genes with a potential for heterologous gene expression. The *pucBa* promoter results in robust, light-induced gene expression useful for bioprocesses which exert high metabolic burden potentially compromising cell viability in absence of photosynthesis-derived energy. The overexpression of alternative nitrogenases will be confirmed by biohydrogen production studies as a direct *in vivo* measure of enzyme activity. In addition to advancing biohydrogen feasibility, the prospect of *R. palustris* as a biotechnological chassis organism is further developed.

**References [Calibri 10]**

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