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Enhanced photo-fermentative hydrogen production by *Rhodobacter capsulatus* with pigment content manipulation

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HIGHLIGHTS

- Recombinant of *Rhodobacter capsulatus* with *cbb3* gene replaced by *pufQ* was obtained.
- The recombinant had reduced light absorption between 300 and 900 nm.
- Photo hydrogen production was improved with reduced pigment mutant.

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ABSTRACT

High content of pigment in purple nonsulfur photosynthetic bacteria hinders its photo-hydrogen production rate under intense light irradiation. In order to alleviate the light shielding effect and improve its photo-fermentative hydrogen production performance, *pufQ*, which is the regulatory gene of bacteriochlorophyll biosynthesis in *Rhodobacter capsulatus*, was cloned and relocated in the genome under *cbb3* promoter by homologous recombination. The UV–vis spectra indicated that the light absorption of the mutant between 300 and 900 nm was reduced. Photo-hydrogen production experiments by the recombinant and wild type strain were carried out in 350 mL photo bioreactors using acetic and butyric acid as substrate. The results showed that the hydrogen production of recombinant with reduced pigment was 27% higher than that of its parental strain, indicating that it is effective on enhancing photo-fermentative hydrogen production by manipulating pigment biosynthesis in purple nonsulfur photosynthetic bacteria.

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1. Introduction

Rhodobacter capsulatus (*R. capsulatus*), which is a kind of purple non-sulfur photosynthetic bacteria (PNSB), can produce hydrogen under nitrogen limited condition with renewable feedstock such as agricultural waste and waste water (Chen et al., 2011; Eroglu and Melis, 2011; Keskin et al., 2011; Li et al., 2011). Nitrogenase is the critical enzyme for hydrogen production of *R. capsulatus*. It reduces protons into hydrogen with electrons and ATP molecules. Light energy, which is essential for hydrogen evolution, is captured by the photosystem. The photosystem apparatus of *R. capsulatus* consists of light harvesting antenna complex I (LHI), light harvesting antenna complex II (LHII) and reaction center (RC). Both LHI and LHII are composed of two membrane spanning α- and β-polypeptides, to which carotenoids and bacteriochlorophyll molecules are bound. The energy is transferred from LHII to a super complex that is formed by a ring of 16 LHI subunits surrounding one reaction center. The structural polypeptides of the photosystem apparatus are coded by *puh*, *puf* and *puc* operons.

The primary pigment of *R. capsulatus* is bacteriochlorophyll (BChl). Enzymes involved in biosynthesis of bacteriochlorophyll are encoded by *bch* gene clusters. These gene clusters are organized in several operons between *puh* and *puf* operons. The polycistronic *puf* operon comprises a regulatory gene *pufQ* and five structural genes *pufBALMX*. The *pufQ* gene is thought to be involved in the regulation of bacteriochlorophyll biosynthesis (Fidai et al., 1995).

Light irradiation condition is quite critical to the hydrogen production performance of PNSB, for it supplies the energy. Because the light intensity decreases quasi-exponentially as the irradiation passage increases in bioreactor (Nakada et al., 1995), incident illumination would become the limiting factor for hydrogen evolution in large scale bioreactors. The microorganisms deep in the bioreactor would be deprived of light, which results in less energy for nitrogenase to produce hydrogen (Guo et al., 2011; Nakada et al., 1995). Meanwhile, the bacteria in the outer space are supplied with excess illumination, and 90% of the energy cannot be utilized. This will cause light damage to the organisms (Melis, 2009;

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