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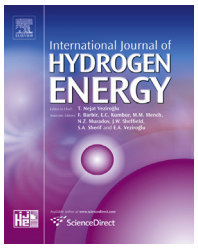

Enhancing photo-fermentative hydrogen production performance of *Rhodobacter capsulatus* by disrupting methylmalonate-semialdehyde dehydrogenase gene

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ABSTRACT

The effects of *mmsA* disruption in *Rhodobacter capsulatus* SB1003 on the pigment content, poly-β-hydroxybutyrate (PHB) content and photo-fermentative hydrogen production were studied. A transposon mutant assigned ZY29 with mutation on *mmsA* and an *mmsA* partially deleted mutant assigned ZYDM9 (*mmsA*$^-$) were obtained. The mutants showed reduced pigment and PHB content, and improved photo-fermentative hydrogen production performance. The hydrogen yield and maximum hydrogen production rate of ZYDM9 were 4675 ± 76 mL/L and 92.4 ± 2.6 mL/(Lh), which increased by 22.8% and 20% compared with that of the WT, respectively. In the meantime, its pigment and PHB content were 20.7 ± 0.1 nmol/mg and 54.5 ± 2.6 mg/g-dcw, which reduced by 34.2% and 43.4% compared with that of WT, respectively. The optimum parameters for hydrogen production were initial pH 7.0, carbon nitrogen ratio (g/g) 3.0:0.5, and light intensity 800 ± 35 W/m^2.
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Introduction

Hydrogen is an attractive alternative fuel for the future because it is environmentally friendly and can be produced from renewable energy sources [1–4]. Photo-fermentative hydrogen production conducted by microorganism is a promising method due to its abundant substrate sources and mild operation conditions [5,6]. *Rhodobacter capsulatus* (*R. capsulatus*) is a model purple non-sulfur photosynthetic bacterium (PNSB), which can produce hydrogen using small organic substrate under photoheterotrophic condition [7–9].

Photo-fermentative hydrogen production with PNSB has been studied intensively in recent years [9–14]. However, low light conversion efficiency is one of the important issues that restrict the development of photo-fermentative hydrogen production. The light energy is captured by the light harvesting complexes. But it is found that the bacteria in the inner space of bioreactor cannot absorb enough light irradiation due to the high amount of bacterial pigment, which blocks the

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penetration of light from outer space. This phenomenon is called the light shielding effect. It has been proved that the light shielding effect was mitigated by decreasing bacterial pigment content, therefore, the hydrogen production yield and rate were enhanced [12,15,16]. A few studies have reported to enhance hydrogen production performance by screening strains with reduced bacterial pigment content. A mutant of *R. capsulatus* SB1003 with disrupted *pucDE* genes showed reduced pigment content by 38%, and increased hydrogen production yield by 50.5% [15]. A *puc*BA-deleted mutant was short of B800–850 complex and its hydrogen production was about two times higher than that of its parental strain [17]. A *puf*Q manipulated recombinant mutant of *R. capsulatus* SB1003 with reduced light absorption between 300 and 900 nm enhanced hydrogen production performance by 27% [18]. Kondo et al. [19] obtained a mutant of *R sphaeroides* RV by UV irradiation. Its bacteriochlorophylls (Bchl *a*) and carotenoids were reduced to 41% and 49% of those from RV, while its hydrogen yield increased by 50% compared with that of the wild type strain. Although many mutants obtained via UV-irradiation method showed higher hydrogen production performance than their wild type [16,20,21], it is difficult to locate the mutated gene that resulted in pigment variation and study the relationship between the mutated gene and hydrogen production. Furthermore, few researches have been reported on the relationship between pigment content and hydrogen production in *R. capsulatus*. The transposon randomly inserts into the genome of WT and the mutations could be easily located by DNA sequencing, therefore, the transposon plasmid is an efficient mutagenesis tool for screening mutants with high hydrogen production performance [12,22].

Methylmalonate-semialdehyde dehydrogenase (MSDH) encoded by *mmsA* gene, which belongs to the aldehyde dehydrogenase superfamily, catalyzes the oxidation of malonate semialdehyde and methylmalonate semialdehyde to produce acetyl-CoA and propionyl-CoA, respectively [23]. In most bacteria, PHB is synthesized in a three-step reaction starting with acetyl-CoA and acetyl-CoA is a crucial precursor in PHB synthesis [24]. Furthermore, it is confirmed in a lot of literature [25,26] that blocking PHB synthesis can improve hydrogen production performance. Therefore, *mmsA* gene might have relationship with PHB and hydrogen production. The *mmsA* gene has been studied in many literature [27–30], however, there was no report on the relationship between MSDH and photo-fermentative hydrogen production in *R. capsulatus* or any other photosynthetic bacteria.

A mutant assigned ZY29 was obtained from wild type strain *R. capsulatus* SB1003 [31] through transposon mutagenesis using plasmid pRL27 [32] combined with high throughput mutant screening method proposed in this work. The transposon insertion site was on methylmalonate-semialdehyde dehydrogenase (*mmsA*) gene located by DNA sequencing. To confirm the effect of *mmsA* gene disruption on hydrogen production, the mutant ZYDM9 was created by partially deleting *mmsA* gene from the genome of WT. The relationship of *mmsA* gene with hydrogen production performance was studied.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *R. capsulatus* SB1003 was used as wild type (WT) strain. The medium MPYE or Sistrom's succinate minimal (MedA) was used for culturing *R. capsulatus* at 35 ± 0.5 °C [33]. For photoheterotrophic growth, the plates were incubated in an anaerobic jar with oxygen consumption gas packs (MGC, Mitsubishi gas chemicalcompany) under about 4500 ± 200 lux (Halogen tungsten lamp) irradiation. *Escherichia coli* (*E. coli*) strains were incubated at 37 ± 0.5 °C in Luria–Bertani (LB) medium supplemented with antibiotics when necessary. Kanamycin at 50 μg/mL or gentamicin at 12 μg/mL was used for *E. coli* strains. Kanamycin at 10 μg/mL orgentamycin at 12 μg/mL was used for *R. capsulatus* strains.

Screening of transposon mutants

Tri-parental cross [34] was conducted by mixing transposon donor pRL27/BW29427, recipient *R. capsulatus* SB1003 and helper pRK2013/S17-1 on MPYE plate at 35 ± 0.5 °C for 24 h incubation. The culture was collected, washed and re-suspended with MedA medium, and then spread on MedA plate supplemented with kanamycin and incubated for two days under photoheterotrophic condition. The mutants with transposon insertion in the genome could show up after two-day incubation. Those mutants that showed light-colored were purified as candidates. These candidates were inoculated into a 96-hole microtiter plate used as micro-bioreactor and sealed with a piece of absorbent filter paper that was soaked with palladium chloride ($PdCl_2$) solution. The 96-hole microtiter plate was incubated under photoheterotrophic condition. Modified MedA was used as medium for photo-fermentative hydrogen production. Palladium chloride reacts with hydrogen to form black palladium, and the mutants with high hydrogen production performance could be identified by the color of filter paper.

Locating mutation site

The genomic DNA extracted from the transposon mutant was digested with enzyme *BamHI* and *Bgl*II, which do not exist in the transposon sequence of plasmid pRL27. Then the digested DNA fragments were purified and self-ligated by T4 DNA ligase (NEB) to generate a new DNA plasmid containing fragments of *R. capsulatus* SB1003 genome and transposon sequence. The obtained new plasmid was transferred into *E. coli* S17-1 for replication and identification. Then the new plasmid was sequenced with primers Tn5-for (CTT GAC GGG ACG GCG GCT TT) and Tn5-rev (CTG ACA TGG GGG GGT ACC GA) that anneal to the transposon sequence. The mutation could be located by blasting the obtained sequence with the genome sequence of WT in the Genebank.

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