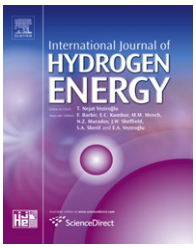
Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he



Hydrogen production properties of *Rhodobacter capsulatus* with genetically modified redox balancing pathways

Yavuz Öztürk [a,*], Abdulmecit Gökçe [a,b], Begüm Peksel [c], Muazzez Gürgan [d], Ebru Özgür [e], Ufuk Gündüz [d], İnci Eroğlu [e], Meral Yücel [d]

[a] TÜBİTAK, Research Institute for Genetic Engineering and Biotechnology, Gebze, Kocaeli 41470, Turkey
[b] Department of Mol. Biology and Genetics, Istanbul Technical University, Istanbul 34469, Turkey
[c] Department of Biotechnology, Middle East Technical University, Ankara 06531, Turkey
[d] Department of Biology, Middle East Technical University, Ankara 06531, Turkey
[e] Department of Chemical Engineering, Middle East Technical University, Ankara 06531, Turkey

ARTICLE INFO

Article history:
Received 12 December 2010
Received in revised form
2 June 2011
Accepted 11 June 2011
Available online 20 July 2011

Keywords:
Rhodobacter capsulatus
Biological hydrogen production
Redox balance
Electron transfer chain
CO_2 fixation
Nitrogen fixation

ABSTRACT

Rhodobacter capsulatus produces molecular hydrogen under the photoheterotrophic growth condition with reduced carbon sources (organic acids). Under this condition, ubiquinol pool is over reduced and excess reducing equivalents are primarily consumed via the reduction of CO_2 through the Calvin–Benson–Bassham (CBB) pathway, the dimethylsulfoxide reductase (DMSOR) system or by the reduction of protons into hydrogen gas with the use of nitrogenase to maintain a balanced intracellular oxidation-reduction potential (redox balance). In order to investigate the effect of redox balancing pathways on nitrogenase-dependent hydrogen production, CO_2 fixation was blocked by inactivating the phosphoribulokinase (PRK) of CBB pathway in wild type (MT1131), uptake-hydrogenase deficient strain (YO3), and cyt cbb_3 oxidase and uptake-hydrogenase deficient double mutant (YO4) strains. The hydrogen production properties of newly generated strains deficient in the CBB pathway were analyzed and compared with wild type strains. The obtained data indicated that, the total hydrogen production was increased slightly in CBB deficient mutant of YO3 and YO4 (4.7% and 12.5% respectively). Moreover, the maximum hydrogen production rate was increased by 13.3% and 12.7% for CBB deficient mutant of MT1131 and YO3 respectively. It was also observed that under the photoheterotrophic growth condition with ammonium as a nitrogen source, PRK deficient strains gave photoheterotrophically competent ammonium insensitive revertants.

1. Introduction

The facultative phototrophic bacterium *Rhodobacter capsulatus* has a number of metabolic pathways to grow under different environmental conditions by performing aerobic respiration, anaerobic respiration, photosynthesis and fermentation [1]. In *R. capsulatus*, anoxygenic photosynthesis is driven by the cyclic electron transfer between the reaction center and the cyt bc_1 complex via the lipid soluble ubiquinone pool and the electron carriers: soluble cytochrome c_2 (cyt c_2) or membrane-bound cytochrome c_y (cyt c_y) [2,3] (Fig. 1). Under photoheterotrophic growth conditions, the oxidation of organic

* *Corresponding author*. Tel.: +90 262 6773329; fax: +90 262 6463929.
E-mail address: yavuz.ozturk@mam.gov.tr (Y. Öztürk).
0360-3199/$ – see front matter
doi:10.1016/j.ijhydene.2011.06.078

Nomenclature

CBB pathway calvin–benson–bassham reductive pentose phosphate pathway
Cyt cytochrome
PRK phosphoribulokinase
Cyt cbb_3 ox cytochrome cbb_3 oxidase
Hup uptake hydrogenase
DMSO dimethylsulfoxide
DMSOR dimethylsulfoxide reductase

acids (such as malate, acetate and lactate) can result in over reduction of the ubiquinone pool. As cyclic photosynthesis requires oxidized ubiquinone as an electron acceptor, excess reducing equivalents, at the level of the reduced ubiquinone pool, are removed by redox balancing systems [4].

The maintenance of intracellular redox poise is achieved by dissipating reducing equivalents through the Calvin–Benson–Bassham (CBB) cycle, the DMSOR system, or the nitrogenase enzyme [5]. *R. capsulatus* assimilate CO_2 via the highly regulated Calvin–Benson–Bassham (CBB) reductive pentose phosphate pathway (Fig. 1). During photo- and chemoautotrophic growth, CO_2 is the sole source of cellular carbon, and maximal levels of the key CBB pathway enzymes; ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) and phosphoribulokinase (PRK) are produced [6]. Photoheterotrophic growth results in much lower yet substantial levels of RubisCO and PRK [7]. Under these conditions, the CBB pathway, rather than serving as a major means for generating organic carbon, plays a main role in redox balance of the cell when carbon substrates are oxidized by allowing CO_2 to serve as an electron sink [5,8]. In *Rhodopseudomonas palustris*, the role of CBB pathway in redox homeostasis was investigated by using ^{13}C-labeled acetate [9]. ^{13}C-labeling experiments highlighted that photoheterotrophic growth is associated with a challenge in maintaining redox balance, and the CO_2-fixing Calvin cycle is not only at the heart of photoautotrophic metabolism but can be a central aspect of photoheterotrophic metabolism as well [9]. Another regulatory mechanism to provide redox balance is the nitrogenase system. Besides its role in nitrogen fixation, it also serves as a redox balancing system during photoheterotrophic growth under limiting nitrogen sources [10,11]. Under such nitrogen limiting conditions, the nitrogenase is activated and the excess reducing equivalents generated by the oxidation of organic acids are

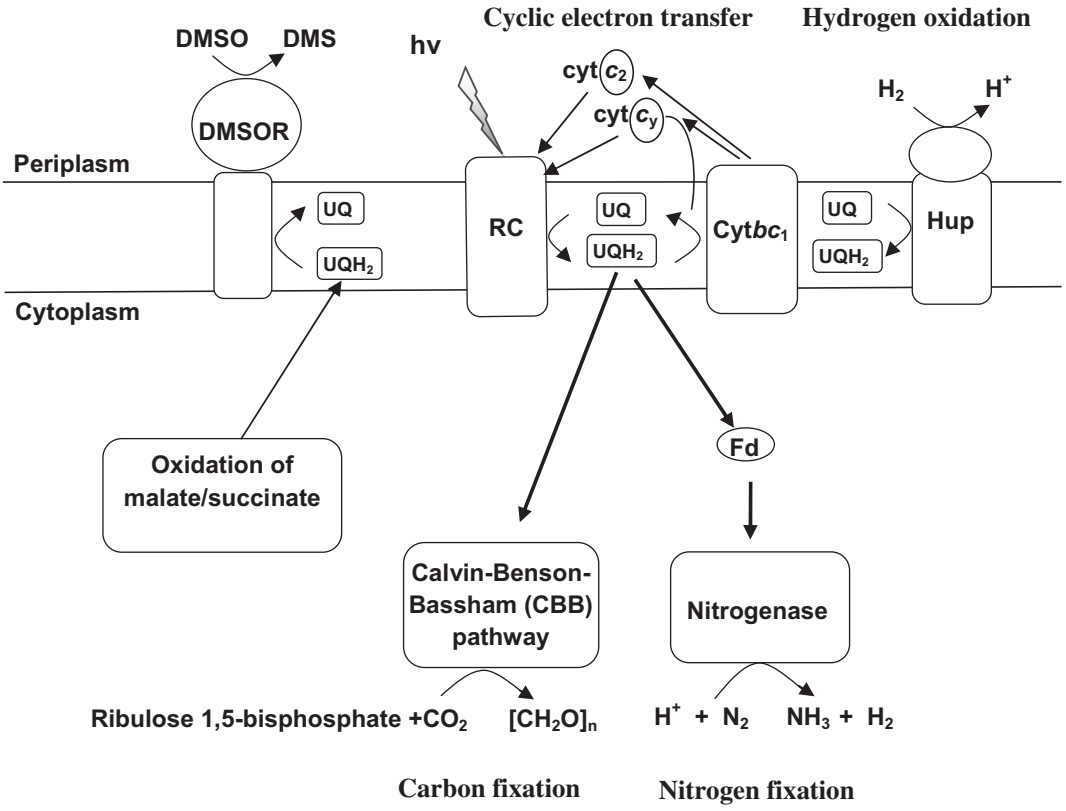


Fig. 1 – Photoheterotrophic electron transport pathway in *R. capsulatus* and model for redox balancing through the ubiquinol pool. Abbreviations: RC, Photosynthetic reaction center; UQH_2, reduced ubiquinol; UQ, oxidized ubiquinone; cyt bc_1, cytochrome bc_1 complex; cyt c_2, soluble cytochrome c_2; cyt c_y, membrane-bound cytochrome c_y; *hν*, light; Hup, uptake hydrogenase; Fd, ferridoxin.; DMS, dimethyl sulfate; DMSO, dimethylsulfoxide; DMSOR, dimethylsulfoxide reductase.