Insertional mutagenesis to select mutants for modified hydrogen photoproduction in

Chlamydomonas reinhardtii

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Introduction

H₂ photoproduction in Chlamydomonas reinhardtii is linked to the presence of an hydrogenase (HydA1) located in the chloroplast and that uses reduced ferredoxin (PetF) to catalyze the reduction of protons to yield H₂ under anaerobic conditions (Figure 1). This production is only transient since O₂ is generated by PSII. In 2000, Melis et al., [1] set up an experimental protocol based on sulfur (S) deprivation, which induces anaerobiosis and allows a long-term hydrogen production by light-exposed C. reinhardtii cultures. In order to enhance understanding of the process, an insertional mutagenesis of Chlamydomonas has been carried out with an hygromycin resistance cassette and 6000 transformants have been generated. The insertional library is screened by Winkler test [2].

Aim

Identify mutants with an attenuated photosynthesis to respiration capacity ratio (P/R ratio) to avoid the stressful sulfur deprivation step in H₂ photoproduction [1].

Results

Winkler test screening

In 2008, Rühle et al., [2] developed a screening protocol called Winkler test that allow to discriminate O₂-evolving (P/R>1) strains and O₂-consuming mutants (P/R<1). It colorimetrically reveals the presence of dissolved O₂ by performing after incubation in the dark (to obtain anaerobiosis) and in the light (to allow O₂ evolution), four oxidoreduction reactions. Attenuated P/R mutant could reach anoxia needed for hydrogenases activity without applying nutritional stress.

Development

• Determination of the parameters to obtain anaerobiosis:
  - One hour in the dark
  - Minimum concentration of 1.5.10⁶ cell/ml (Figure 2)
  - In order to easily check the cell concentration, optical density is measured at 750nm. An A₄₀₀ value between 0.3 and 0.6 is required to avoid false negatives.
  - WT+DCMU is a valid negative control; TAP and WT are valid positive controls (Figure 2).
  → These optimizations allow to screen about 2x250 transformants/day.

About 6000 transformants have been already screened (Figure 3) and about twenty mutants corresponding to the phenotype of interest have been selectionned (Figure 4).

Outlook

O₂ evolution curves must be analyzed in details to confirm the modified P/R ratio (Figure 5).

In attenuated P/R mutant, the compensation point is reached later (shift to the right, to higher light intensity). Such mutants would be able to reach anaerobiosis without dropping the PSII.