Insertional mutagenesis to select mutants for modified hydrogen photoproduction in *Chlamydomonas reinhardtii*

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Introduction

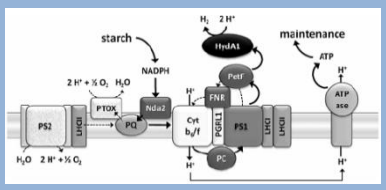

Figure 1: Schematic of photosynthesis electron transport during H_2 production by S-deficient *C. reinhardtii* cells [3].

H_2 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 H_2 under anaerobic conditions (Figure 1). This production is only transient since O_2 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 H_2 photoproduction [1].

Results

Winkler test screening

In 2008, Rühle *et al.*, [2] developed a screening protocol called Winkler test that allow to discriminate O_2-evolving (P/R>1) strains and O_2-consuming mutants (P/R<1). It colorimetrically reveals the presence of dissolved O_2 by performing after incubation in the dark (to obtain anaerobiosis) and in the light (to allow O_2 evollution), 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 $1.5.10^6$ cell/ml (Figure 2)
- In order to easily checck the cell concentration, optical density is measured at 750nm. An A_{750} 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.

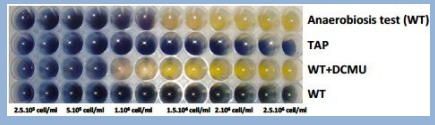

Figure 2: Determination of the Winkler test conditions.

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

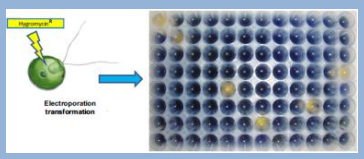

Figure 3: Colonies from the insertional library were transferred to thee 96-well plates and screened by Winkler test. Wells that remain colorless correspond to negative controls (WT+DCMU).

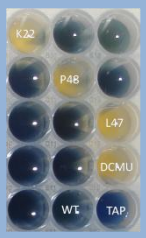

Figure 4: Identification of three P/R<1 mutants (K22, P48, L47).

Outlook

O_2 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.

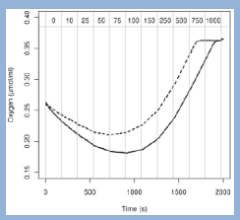

Figure 5: Exemple of O_2 evolution curves between a WT strain (- - -) and a P/R<1 mutant (——).

[1] Melis, A., L. Zhang, et al., (2000). "Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*." Plant Physiol 122(1):127-136.

[2] Rühle, T., et al., (2008). "A novel screening protocol for the isolation of hydrogen producing *Chlamydomonas reinhardtii* strains". BMC Plant Biology 2008, 8:107.

[3] Hemschemeier. A., Happe. T., (2011). "Alternative photosynthetic electron transport pathways during anaerobiosis in the green alga *Chlamydomonas reinhardtii*." Biochim Biophys acta. 2011 [Epub ahead of print].