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

Damien Godaux[1], Barbara Emonds-Alt[1], Bénédicte Vertruyen[3], Philippe Compère[4], Pierre Cardol[1], Fabrice Franck[2], Claire Remacle[1]

[1] Laboratoire de Génétique des microorganismes, [2] Laboratoire de Biochimie végétale, Institut de Botanique B22, [3] Laboratoire de Chimie inorganique structurale, [4] Laboratoire de morphologie ultrastructurale, Institut de Chimie B6C, Université de Liège, B-4000 Liège, Belgique.

Introduction

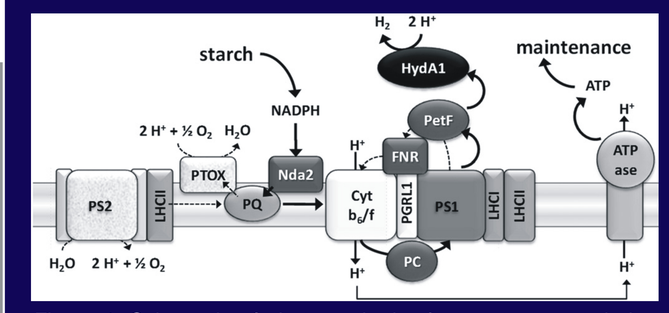

Figure 1: Schematic of photosynthetic electron transport during H_2-production by S-deficient *C. reinhardtii* cells [4].

H_2 photoproduction in *C. reinhardtii* is linked to the presence of an hydrogenase (HydA1) located in the chloroplast and that uses reduced ferrodoxin (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 about 4500 transformants have been generated. The insertional library is screened by 2 different protocols.

Aims

1. 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] .
2. Develop sensitive chemochromic sensor films which turn in blue in presence of H_2 in order to isolate mutants whith attenuated levels of H_2 photoproduction.

Results

1 Winkler test screening

Winkler test [2] allows to discriminate O_2-evolving (P/R>1) strains and O_2-consuming mutants (P/R<1) by performing after incubation in the dark (to obtain anaerobiosis) and in the light (to allow O_2 evolution),4 oxidoreduction reactions which colorimetrically reveal the presence of dissolved O_2 .

<u>Development</u>

- Determination of the parameters to obtain anaerobiosis:
  - 1 hour in the dark
  - minimum concentration of $1.5.10^6$ cell/ml (Figure 2)
- In order to easily check 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

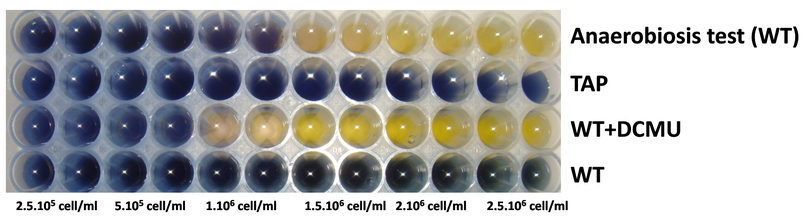

Figure 2: Determination of the Winkler test conditions.

About 2000 transformants have been already screened (Figure 3).

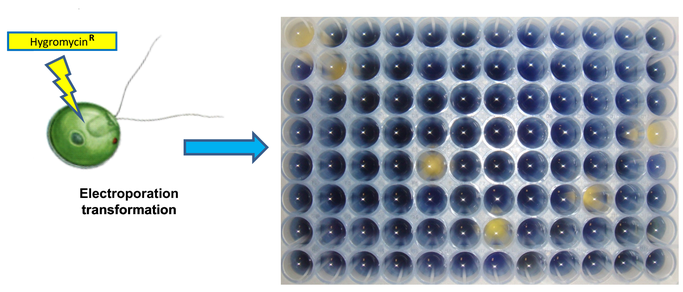

Figure 3: Colonies from the insertional library were transferred to 96-well plates and screened by Winkler test. Wells that remain colorless correspond to negative controls (WT+DCMU).

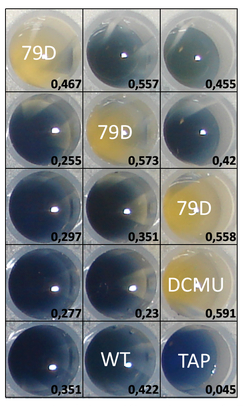

Figure 4: Identification of 79D mutant. In black, absorbance measured at 750nm.

➠ Isolation of 79D mutant (Figure 4)

Chemochromic film 2

A chemochromic sensor WO3 film [3] has been realized by dip-coating. Sensitive to H_2, it turns in blue in presence of H_2 (Figure 6). A tungsten thickness of 500 to 600nm is required to allow the screening procedure (Figure 7). Now, the screening of the insertional library is on the brink of start.

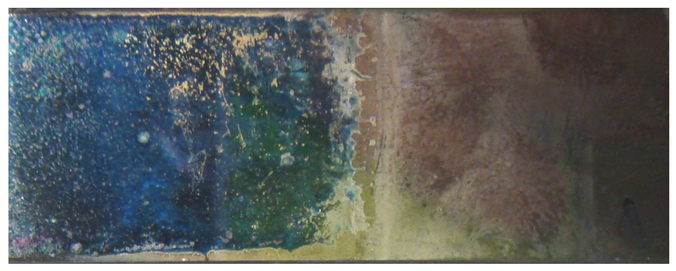

Figure 6: Chemochromic film turns in blue in presence of H_2.

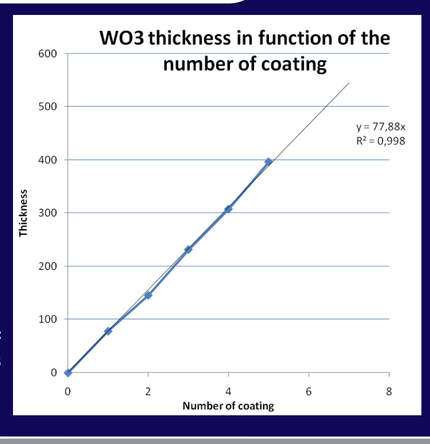

Figure 7: Thickness of chemochromic sensor film, measured by ellipsometry, is proportional to the number of coating.

The comparison of O_2 evolution curves between WT and 79D shows that the compensation point is reached later for 79D (Figure 5).

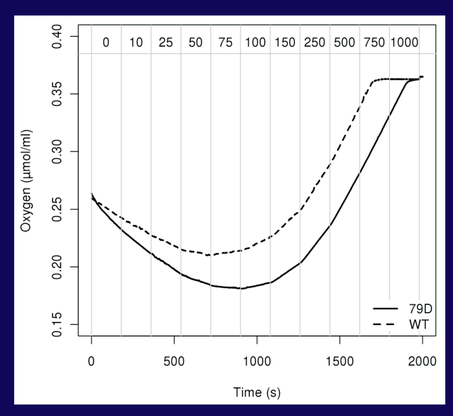

Figure 5: A compensation point shifted to the right means that 79D would be able to reach anaerobiosis without dropping the PSII.

➠ 79D seems to be a good candidate for photoH_2 production in normal growth condition

References :

[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] Posewitz, M.C., (2005). “Identification of genes required for hydrogenase activity in *Chlamydomonas reinhardtii*.” Biochemical Society Transactions Volume 33, part 1.

[4] 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].