Introduction

The unicellular green alga C. reinhardtii can grow either phototrophically with CO₂ as the sole carbon source, heterotrophically by consuming acetate in the dark, and mixotrophically by using both carbon sources in the light. Despite significant knowledge gained on acetate metabolism, the genes coding for acetyl-CoA synthase, acetate kinase, isocitrate lyase, malate synthase and malate dehydrogenase etc. are characterized by a close gene structure (Fig 1) and very high similarity in their coding sequences (CDS) except for a clear distinction at the N-terminus amino acid sequences (Fig 2).

A reverse functional genomics approach by using artificial micro RNA (amiRNA) gene silencing was adopted to target the five genes one-by-one. Used alone, ~160 transformants were generated for each amiRNA construct and their characterization is ongoing. Further characterization of the mutants will follow to have an understanding of the gene function in the acetate metabolism.

Results

Transcripts quantification

Almost all transcripts were downregulated when cells were exposed to high light. GFY1 and GFY2 were especially expressed in anoxic condition were normally acetate is excreted outside the cell. In conclusion, our preliminary data suggest differentiated roles of the acetate transporters into acetate metabolism and/or eventually a different subcellular localization.

GFY genes display a strictly correlation with the expression of genes involved in carbon metabolism, especially related to the primary biochemical steps of acetate assimilation, i.e. acetyl-CoA synthase, acetate kinase, isocitrate lyase, malate synthase and malate dehydrogenase etc..

Co-expression

Methods

Cells of wall-less strain 325.3 (wt-137 mt+) were cultivated in six different conditions:

- **TAP_L** Control condition (acetate 16.65 mM, 60 uE m⁻² s⁻¹ PAR)
- **TMP_L** Autotrophy (minimal medium, 60 uE m⁻² s⁻¹ PAR)
- **TAP_LL** Low light (acetate 16.65 mM, 15 uE m⁻² s⁻¹ PAR)
- **TAP_HL** High light (acetate 16.65 mM, 260 uE m⁻² s⁻¹ PAR)
- **TAP_D** Heterotrophy (acetate 16.65 mM, dark)

Anaerobiosis 6h of anaerobic adaptation by N2 purging

Conclusions

The GFY1/FUN34/yaH genes found in C. reinhardtii are characterized by very high similarity in their coding sequences, letting us to think for a redundant role. However, the divergent amino acid composition at the N-terminus and the distinct expression under the different culture conditions tested point to a different situation. In conclusion, our preliminary data suggest differentiated roles of the putative acetate transporters into acetate metabolism and/or eventually a different subcellular localization.

References: