Use comparative proteomics on a selected microalgae provides candidates for biofuel production.

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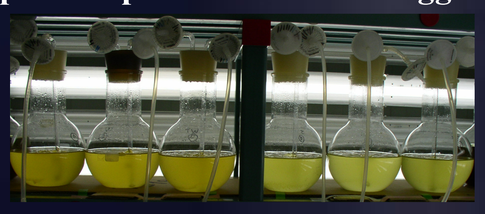
CONTEXT:

Understanding microalgal lipid accumulation is of major interest for feedstocks, food and biofuel production. Although studies have been performed in model species, to this day it is critical to understand through particular mechanisms of biotechnological species.

Tisochrysis lutea is traditionally used for aquaculture feed and presents advantages for poly-unsaturated fatty acids and neutral lipid production. A lipid over-accumulating selected mutant (S2M2) was previously obtained by UV mutation and flow cytometry selection. The identification of proteins involved in the up accumulation of neutral lipids under nitrogen limitation would provides insights for biotechnological applications such as biofuel production.

✓**Up accumulation of neutral lipids in S2M2 mutant is enhanced by nitrogen limitation.**

✓**Lipids droplets in S2M2 are bigger and more numerous**



Sampling for proteomics

Fig 1 : Boths strains were cultured in triplicate in nitrogen limiting batch.

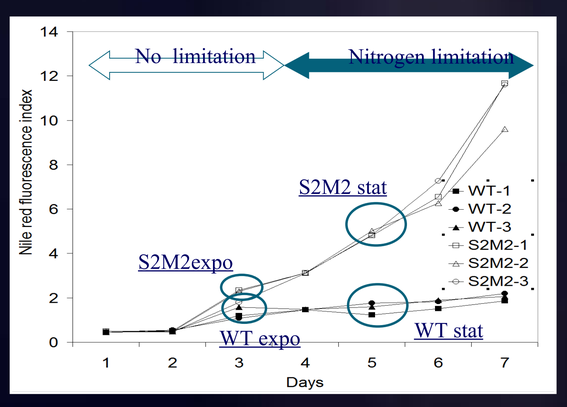


Fig 2 : Neutral lipid accumulation

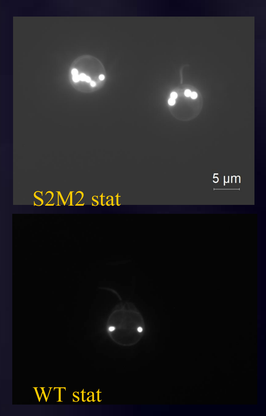


Fig 3 : Lipid droplets stained with Nile Red

✓Tentative mapping of metabolisms of identified proteins

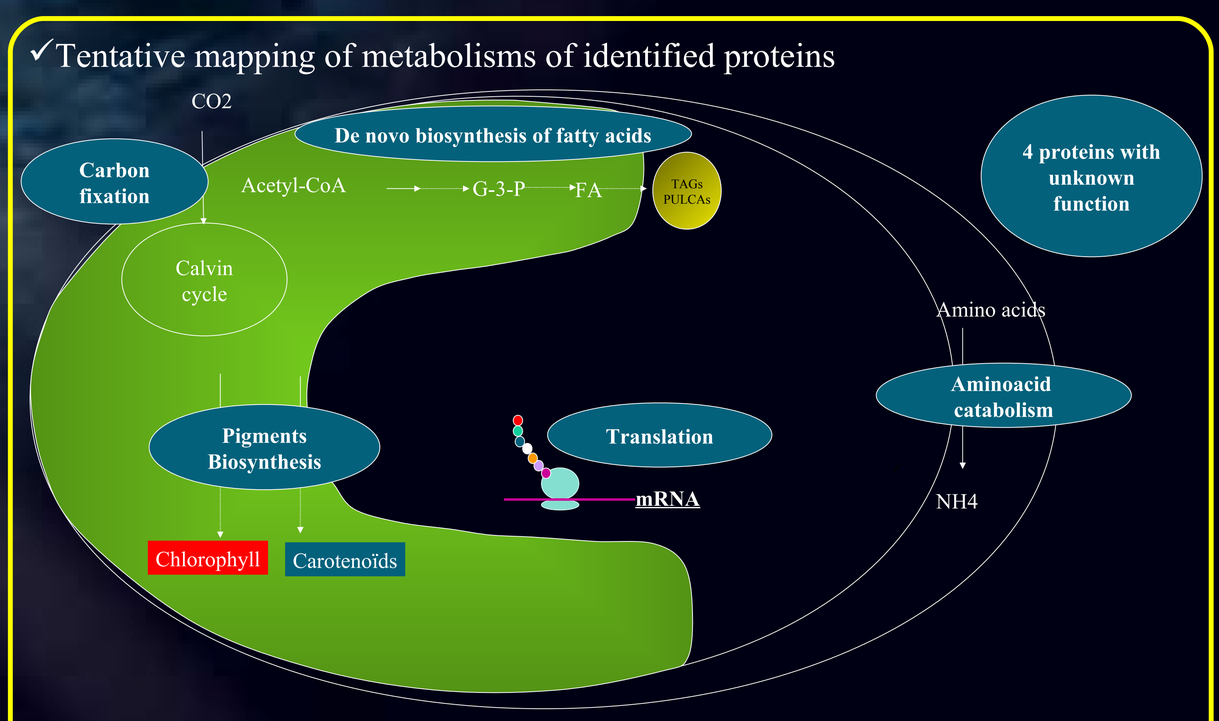


Fig. 7 : Metabolisms affected by nitrogen limitation

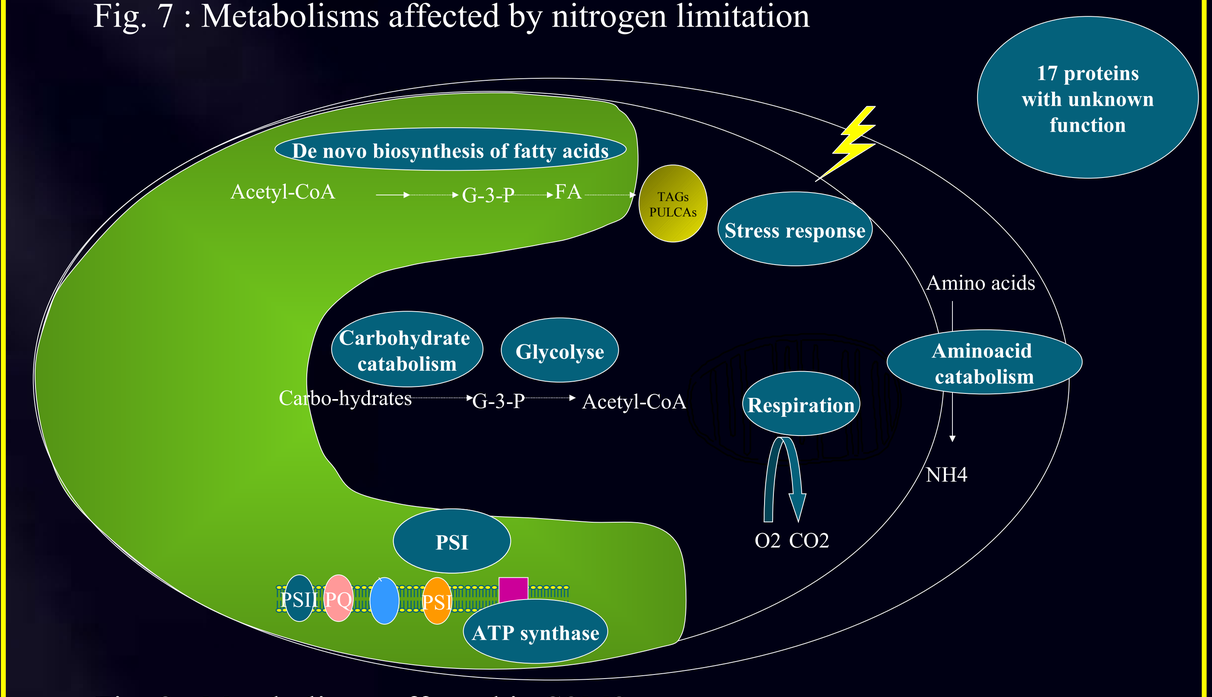


Fig. 8 : Metabolisms affected in S2M2

✓Comparative proteomics by 2-DE reveals specific behaviours for each strains during lipid accumulation

Fig. 4 : x24 2-DE gels (pH4-7 ; 12% acrylamide)

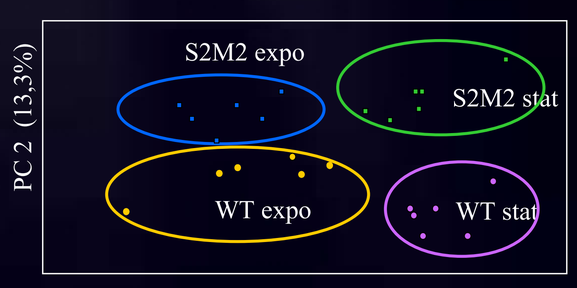
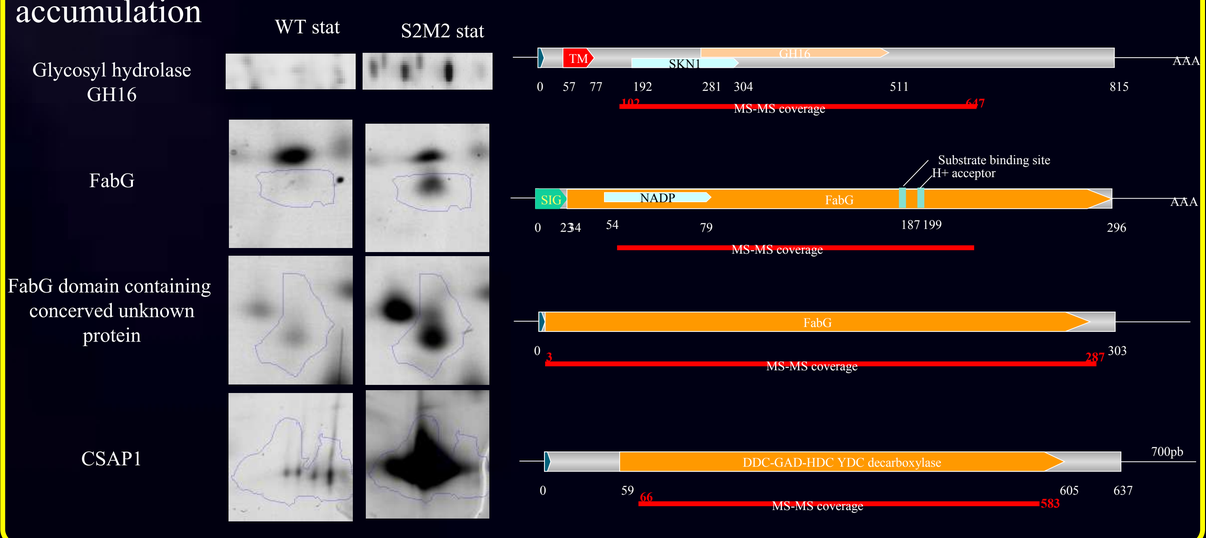


Fig. 5 : PCA on normalized spot volume (1850 spots x 24 gels)

fold > 2 ; q < 0.05 ; P>80%	Nb of spots
Up in S2MS	25
Down in S2M2	19
Up at stat phase	5
Down at stat phase	15

✓Four proteins were selected for beeing putatively involved in lipid up accumulation



✓ Genomic data are of major importance for protein identification in non model species.

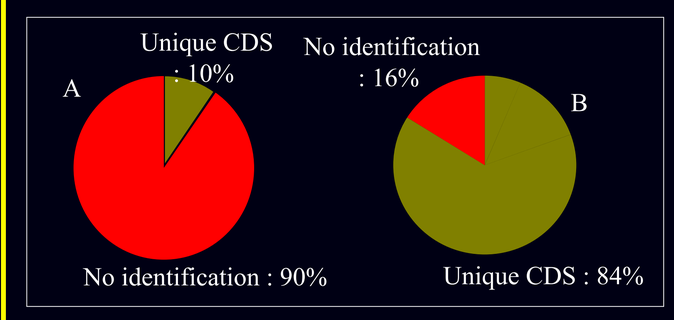


Fig. 6 : MS-MS and Mascot analysis on 57 spots (A) before \ (B) after transcriptome sequencing

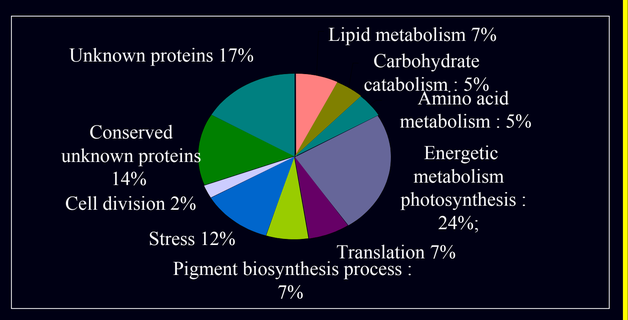


Fig. 6 : Functionnal anotation

✓**33 % of identified proteins have unknown function.**

Conclusions:

Proteomics on non model species is limited by the lack of genomic data and the lake of functionnal annotation.

This work is the first comparative proteomic analysis of *Tisochrysis lutea*. The results highlight mecanisms affected nitrogen limitation and by strain mutation-selection. They reveal a set of proteins potentially involved in the up accumulation of neutral lipids. They include proteins involved in 1) carbohydrates catabolism, 2) de novo biosynthesis of fatty acids and 3) carbon homeostasy. The redirecting of carbon from carbohydrates to fatty acid biosynthesis could be of great importance.

Numerous proteins of unknown function have been identified and should be the object of futures target studies.

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