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Identification of transcription factors involved in the phenotype of a domesticated oleaginous microalgae strain of *Tisochrysis lutea*

S. Thiriet-Rupert[a,*,1], G. Carrier[a], C. Trottier[a,2], D. Eveillard[b], B. Schoefs[c], G. Bougaran[a], J.-P. Cadoret[a,d], B. Chénais[c], B. Saint-Jean[a]

[a] IFREMER, Physiology and Biotechnology of Algae Laboratories, Rue de l'Ile d'Yeu, 44311, Nantes, France
[b] LS2N, UMR6004, CNRS, Université de Nantes, Ecole Centrale de Nantes, IMTA, Nantes, France
[c] MIMMA, Metabolism, Engineering of Microalgal Biomolecules and Applications (MIMMA), Mer Molécules Santé, IUML, FR3473 CNRS, UBL, University of Le Mans, Le Mans, France
[d] Greensea SA, Meze, France

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ABSTRACT

Microalgae are an outstanding source of high value products with applications in food, feed or biofuel production. Among these promising organisms, the haptophyte *Tisochrysis lutea* is commonly used as a feed for shellfish and shrimps in aquaculture. Its capacity to produce high amounts of docosahexanoic acid and storage lipids is also of economic interest for nutrition and energy production. Consequently, understanding its lipid accumulation under nitrogen deprivation is of major interest.

Here, we aimed to identify Transcription Factors (TFs) involved in the establishment of the mutant phenotype of the 2Xc1 strain of *T. lutea*, which accumulates double the quantity of storage lipids under nitrogen deprivation than the wild type strain (WTc1). Strains were grown in chemostats and subjected to different nitrogen availability (limitation, repletion and depletion). Using RNA-seq data, the differentially expressed genes (DEGs) between strains were identified and summarized as a co-expression network to pinpoint putative major TFs in mutant phenotypes. This analysis was followed by a complementary Weighted Gene Correlation Network Analysis in order to classify genes based on their relative importance to mutant phenotype features, regardless of annotation biases due to the lack of functional annotation of the *Tisochrysis lutea* draft genome. This network-like strategy allowed the identification of seven TF candidates related to key functions in the mutant strain compared with WTc1. In particular, MYB-2R_14 and NF-YB_2 TFs are related to photosynthesis, oxidative stress response and triacylglycerol synthesis. GATA_2, MYB-rel_11 and MYB-2R_20 TFs are likely to be related to nitrogen uptake or carbon and nitrogen recycling, feeding carbohydrate synthesis in the form of chrysolaminarin. Finally, a q-RT-PCR approach further characterized the role of MYB-rel_11 and MYB-2R_20, revealing an expression pattern dependent on nitrogen availability.

1. Introduction

Although they account for < 1% of the photosynthetic biomass of our planet, the phytoplankton living in the photic zone of the ocean are responsible for around 45% of annual net primary productivity [1]. Among them, microalgae remain corner-stone species for aquatic ecosystem behaviours. Not only are they at the base of trophic chains, but are also key players in nitrogen, phosphorus or carbon biogeochemical cycles [2]. The number of microalgae species is thought to be as many as 70,000 [3] and evenly distributed in the eukaryotic tree [4]. This broad diversity is a source of a variety of high value compounds that, together with the capacity of microalgae to achieve a greater biomass production from light than terrestrial plants, makes microalgae of outstanding biotechnological interest [5–9] and has led to the design of many studies to enhance their production of high value products [10–13]. Among microalgae, haptophytes are distributed worldwide and participate greatly in global climate regulation as a carbon sink [14]. They produce alkenones, long-chain unsaturated methyl and ethyl

* Corresponding author.
E-mail addresses: sthiriet@uliege.be (S. Thiriet-Rupert), gregory.carrier@ifremer.fr (G. Carrier), camille.trottier@univ-nantes.fr (C. Trottier), damien.eveillard@univ-nantes.fr (D. Eveillard), benoit.schoefs@univ-lemans.fr (B. Schoefs), gael.bougaran@ifremer.fr (G. Bougaran), jeanpaulcadoret@greensea.fr (J.-P. Cadoret), benoit.chenais@univ-lemans.fr (B. Chénais), bruno.saintjean@ifremer.fr (B. Saint-Jean).
[1] Present address: InBioS-PhytoSystems, Functional Genomics and Plant Molecular Imaging, University of Liège, Liège, B-4000, Belgium.
[2] Present address: LS2N, UMR6004, CNRS, Université de Nantes, Ecole Centrale de Nantes, IMTA, Nantes, France.

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n-ketones (C37 to C40), which are exclusive to five haptophytes genera [15,16] and widely used as biomarkers for the reconstruction of marine paleoclimates [16]. The non-calcifying haptophyte *Tisochrysis lutea* is used in aquaculture as a feed for shellfish and shrimps because of its attractive fatty acid content [17]. Its production of docosahexanoic acid (DHA) and storage lipids is also of interest from nutritional and energy production perspectives [18–20]. In such a biotechnological context, studying microalgae mutant strains favourable for accumulation of industrial compounds is of primary interest. In particular, studying these strains can help us to better understand the mechanisms underlying interesting phenotypes. Because these key mechanisms are the consequence of genome expression modulation in which Transcription Factors (TFs) are critical players, we sought to identify TFs involved in a given phenotype, as well as their related genes, using network analysis.

This work focuses on the haptophyte microalga *T. lutea*, for which a mutant strain over-accumulating storage lipids (*T. lutea* 2Xc1) was selected by UVc random mutagenesis followed by flow cytometry selection for cell storage lipid content [21]. Previous proteomic and transcriptomic analyses revealed that wild type (WTc1) and mutant strains behave differently during early phases of nitrogen starvation, and suggested that proteins involved in carbon homeostasis, lipid metabolism and carbohydrate catabolism are likely involved in lipid accumulation [22,23]. A previous study investigated the metabolic strain specificities following fine-tuned changes of nitrogen availability, confirming the neutral lipid over-accumulation of 2Xc1, as well as an increase of its carbon accumulation capacity due to an increase in cell carbohydrate content [24] (Fig. 1).

To get further insight into the regulation of these metabolic changes, we analyzed the transcriptomic data generated during the experiment in [24]. Given the crucial role of TFs to monitor metabolism and their recent identification in *T. lutea* [25], we aimed to identify those TFs involved in the mutant phenotype of the 2Xc1 strain of *T. lutea*, using gene co-expression network construction and analysis. To characterize the putative role of these TF candidates in the establishment of the mutant phenotype, the functional annotation of their co-expressed genes was carried out. To avoid drawbacks due to sparse annotation, phenotypically relevant genes were selected, regardless of their annotation, for their links within network analysis to phenotypic features such as cell storage lipid or carbohydrate content dynamics over time. Such a selection of phenotypic parameters was intended to provide guidance to emphasize genes of interest but also the corresponding TFs and their putative roles in the mutant phenotype. Finally, for the sake of experimental validation, a RT-q-PCR approach was carried out to further characterize the expression profile of two pinpointed TF candidates.

2. Material and methods

2.1. Culture conditions and treatments

Following a previous study protocol [24], clones of *Tisochrysis lutea* CCAP 927/14 wild type (WTc1) and mutant CCAP 927/14 (2Xc1) strains were considered. The WTc1 and 2Xc1 clones were grown for 85 days in chemostat at a 0.5 d^{-1} dilution rate in modified Walne's medium containing 125/125 µM N:P ratios in 10-L photobioreactors illuminated with continuous light (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and maintained at 27 °C and pH 7.3. The dilution rate was periodically checked by weighing the outgoing medium. Three nitrogen spikes were made at days 20, 43 and 83. Each spike consisted in the injection of 3.5 mmoles of $NaNO_3$ into the 10 L of culture.

Once the nitrogen-limited culture reached a steady state characterized by constant physiological parameters, the $NaNO_3$ injection was made [24]. Such nitrogen repletion conditions induced an increase of cell concentration (CC) and particulate carbon (PC). A N/C ratio increase indicates an uptake of microalgae nitrogen. Then, CC, PC and the N/C ratio maintained a high level and the lack of nitrogen available in the culture medium induced a decrease of this ratio, characteristic of nitrogen depleted conditions. Then, the culture reached a new steady state due to the dilution rate of the chemostat [24]. All these physiological parameters were taken from [24]. The lipid data were obtained using Nile red staining as advised in [26]. Total carbohydrate data were obtained following [27].

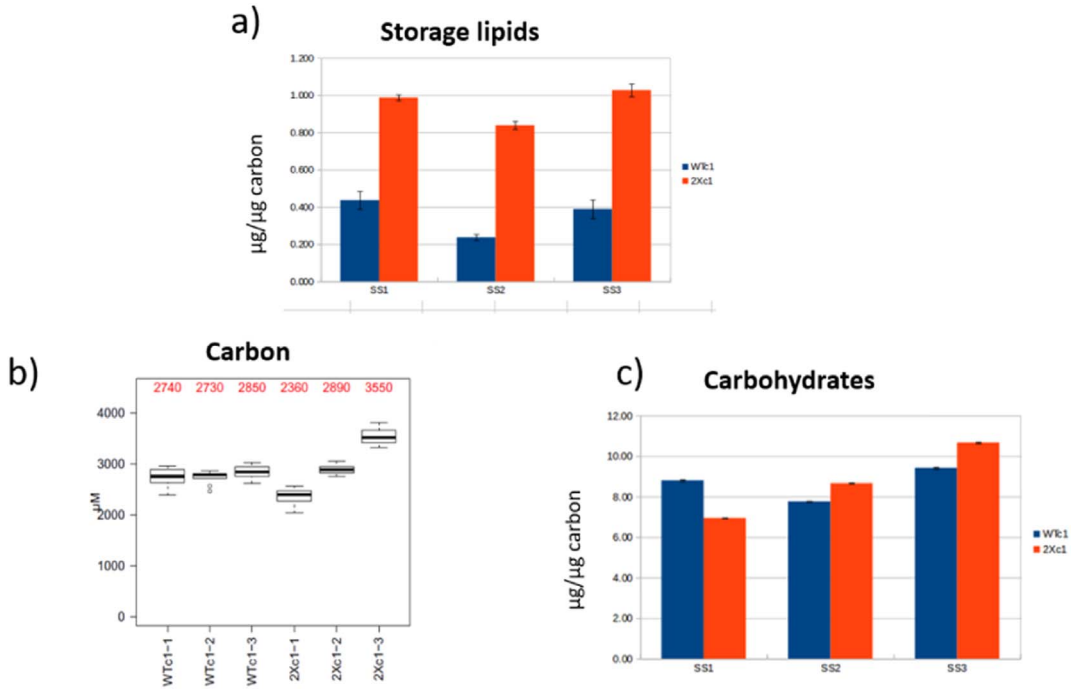

Fig. 1. The physiological state of the two strains was evaluated for the three consecutive steady states (SS1, SS2 and SS3 on each graph) during 85 days of culture [24]. The storage lipid over-accumulation of the 2Xc1 strain was confirmed (a). An increase of cellular carbon of the 2Xc1 strain after each nitrogen spike (b) was shown to be correlated to the cell carbohydrate content (c).