

Enhancing diatom lipid production through metabolic engineering, first phases

Matthijs Michiel^{1,2,3}, Michele Fabris^{1,2,3}, Gino Baart^{1,2,3}, Alain Goossens^{1,2} and Wim Vyverman³

¹ Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Ghent, Belgium
E-mail: mimat@psb.vib-ugent.be

² Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, B-9052 Ghent, Belgium

³ Department of Biology, Ghent University, Krijgslaan 281, B-9000 Ghent, Belgium

The biological productivity of marine heterokonts, and specifically diatoms, is one of the major contributors to the marine foodweb. In growth limiting conditions diatoms store energy and carbon in the form of lipids. Despite the fact that part of these lipids are nutritionally beneficial omega-3 polyunsaturated fatty acids, they are not yet directly employed for human or animal consumption. One of the main reasons is the limited diatom lipid productivity which limits their economic potential. In this project we will attempt to remedy this problem through genetic engineering to increase lipid productivity. We are aiming to identify the key regulator genes that drive lipid synthesis. Our methodology is based on functional genomics and focuses on the model diatom *Phaeodactylum tricornutum*, as its genome has been sequenced and stable transformation is possible. We are using RNA-seq to sequence the transcriptome of *P. tricornutum* during stress conditions that induce lipid synthesis and we will compare these transcriptome profiles with an exponentially growing control. In this way we map the metabolic reprogramming that cells undergo during the switch from growth towards carbon storage and lipid production in particular. Since we are mainly interested in the regulators of this program and, to a lesser degree, in the enzymes actually effectuating this change we are performing our analysis during the first 24 hours of growth under limiting conditions. In the second phase of the project we will attempt to activate this program during conditions that favor exponential growth. It is our goal to get the diatoms to continue growing while also investing into lipid production. The first results show that *P. tricornutum* lipid productivity can be enhanced above baseline conditions through overexpression of genes as proven by ectopic overexpression of an endogenous malic enzyme isoform. Preliminary transcriptome analysis using cDNA-AFLP shows that 2-5% of genes respond to growth adverse growth conditions. Furthermore our detailed metabolic analysis shows that there are quantitative changes detectable in chrysolaminaran and lipid storage products. These changes appear to be coupled to cell cycle progression. While commercially relevant production has not yet been achieved, it is clear that important insights into physiological stresses will be gained through this project.