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Research paper

# Effect of environmental conditions on various enzyme activities and triacylglycerol contents in cultures of the freshwater diatom, *Asterionella formosa* (*Bacillariophyceae*)

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ABSTRACT

A detailed analysis of triacylglycerols (TAGs) contents, fatty acid patterns and key enzyme activities in the freshwater diatom *Asterionella formosa* was performed under various conditions, including nitrate, iron and silicon limitation (stress conditions), or bicarbonate and phytohormones supplementation (stimulation conditions). Of all the conditions tested, the addition of bicarbonate produced the greatest increase (5-fold) in TAGs contents compared to the control while the biomass increased. The addition of phytohormones also allowed a significant increase in TAGs of about 3-fold while the biomass increased. Silicon, unlike iron and nitrate limitation, also triggered a significant increase in TAGs contents of 3.5-fold but negatively affected the biomass. Analysis of fatty acid profiles showed that the mono-unsaturated C16:1 fatty acid was the most abundant in *A. formosa*, followed by C16:0, C14:0 and eicosapentaenoic acid (EPA; C20:5 n-3). EPA levels were found to increase under nitrate and iron limitation. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoribulokinase (PRK), phosphofructokinase (PFK), glucose-6-phosphate dehydrogenase (G6PDH) and malate dehydrogenase (MDH) activities differed with growth conditions. Most enzymes were up-regulated in stimulated cells while in the case of stressed cells, the pattern of activities was more variable. Detailed analysis of all enzyme activities showed that the most important enzyme among those tested was GAPDH which could be a good candidate for genetic engineering of high lipid-producing algae. This study provides a better understanding of key enzymes and biochemical pathways involved in lipid accumulation processes in diatoms.

## 1. Introduction

Diatoms (*Bacillariophyceae*) are a diverse group of eukaryotic unicellular microalgae that are able to use solar energy to combine $CO_2$ with water for the production of biomass and are among the key organisms removing the greenhouse gas $CO_2$ from the Earth's atmosphere [1]. These photosynthetic organisms evolved during the Mesozoic era (251–65 million years ago) and are now major actors in the cycling of elements in oceans [2]. They contribute up to 40% of marine productivity [2,3] but are also frequent and abundant in most freshwaters where they are often the dominant phylogenetic group.

Attention has focused on biofuel production by microalgae, including diatoms, because they have a higher photosynthetic efficiency, biomass production and faster growth than other energy crops. They also do not compete with food crops for resources or space [4–8]. They store energy and carbon in the form of neutral lipids (*i.e.*, triacylglycerols, TAGs [9,10]); that can be easily

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*Abbreviations:* DTT, reduced dithiothreitol; DM, diatom medium; EDTA, ethylene diamine; tetra-acetic acid, GAPDH; glyceraldehyde-3-phosphate dehydrogenase, G6PDH; glucose-6-phosphate dehydrogenase, MDH; malate dehydrogenase, NS; not significant, OD; optical density, OPP; oxidative pentose phosphate pathway, PFK; phosphofructokinase, PRK; phosphoribulokinase, Rubisco; ribulose bisphosphate carboxylase oxygenase, TCA; tricarboxylic acid cycle, GA; gibberellic acid, OD; optical density, BPGA; 1,3-bisphosphoglyceric acid, TLC; thin layer chromatography, GC; gas chromatography, FAMEs; fatty acids to fatty acid methyl ester, FID; flame ionization detector, EPA eicosapentaenoic acid; LC-PUFA, long chain polyunsaturated fatty acids; DHA, docosahexaenoic acid; SAFA, saturated fatty acids; ACCase, acetyl CoA carboxylase; TAGs, triglycerides.

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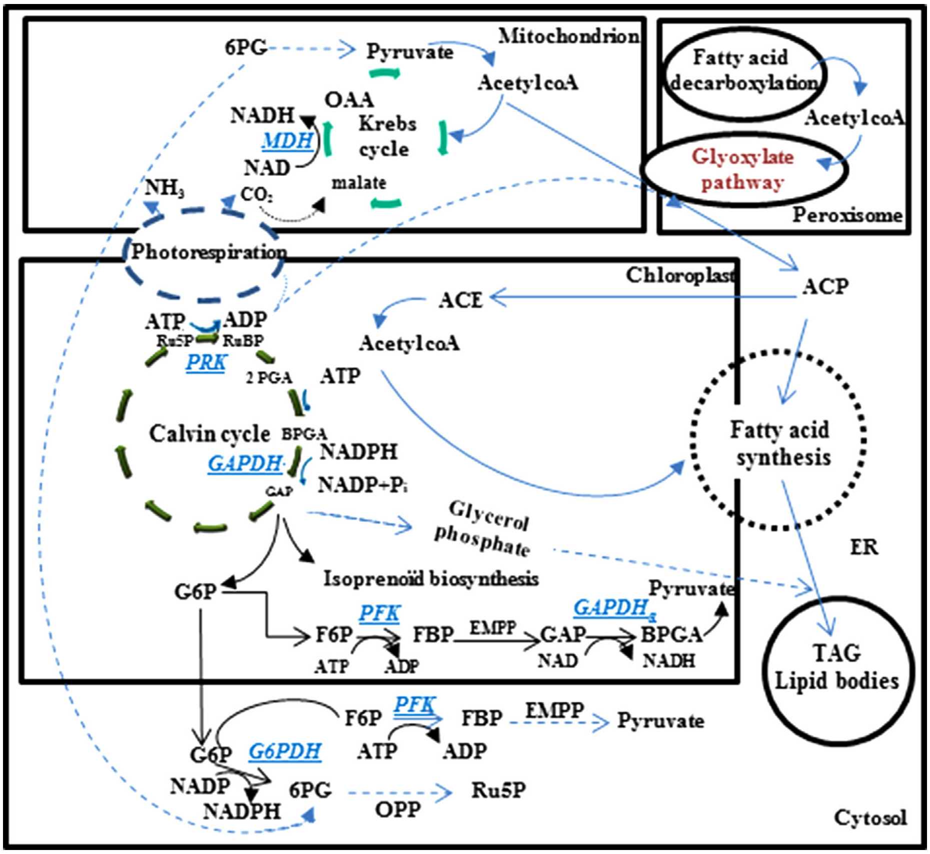

**Fig. 1.** Schematic representation of metabolic pathways in a diatom. For sake of clarity, urea cycle and 1,3-β-glucan synthesis that occur in the mitochondrion and in the vacuole respectively, are not shown. Nucleotide synthesis that is located in the cytosol and not in the plastid like in higher plants is also not shown. EMPP and OPP stand for Embden–Meyerhof–Parnas and oxidative pentose phosphate pathway respectively. The metabolites are ACP: acetyl phosphate; ACE: acetate; F6P: fructose-6-phosphate; FBP: fructose-1,6-bisphosphate; GAP: glyceraldehyde-3-phosphate; G6P: glucose-6-phosphate; OAA: oxaloacetate; 6PG: 6 phosphogluconate; PGA: 3-phosphoglycerate; Ru5P: ribulose-5-phosphate; RuBP: ribulose-1,5-bisphosphate; TAGs, triglycerides. In blue are annotated, the enzymes that have analysed in this study. GAPDH and GAPDH$_a$ stand for chloroplast and glycolytic GAPDH, respectively. Adapted from Refs. [20,65–69].

converted to biodiesel by methanolysis. Since microalgae in general are considered as promising for the production of biofuels, a number of species have been studied for their growth and lipid content [11,12]. Some species can contain up to 60% oil on a dry biomass basis [13].

There is a broad range in TAG yields among species [14], but it is clear that the optimal production of TAGs occurs in diatoms under conditions of environmental stress, such as nutrient deficiency or low temperature. The decrease of growth rate is due to minimizing nutrients and therefore the expense of carbon source can be channelled into TAG synthesis [15,16]. However recently, adding bicarbonate salts has been shown to increase lipid accumulation without biomass decrease in *Phaeodactylum tricornutum* [17]. Transcript levels of some genes involved in the metabolism of bicarbonate-treated diatom have been analysed [18] but no enzyme activity has been measured. Because of the evolutionary history of diatoms [19], their genomes and their metabolism result rather from a complex mixture of highly divergent sources such as bacteria, green and red algae. Similar to green algae and terrestrial plants, diatom genomes contain genes encoding for the essential enzymes of the central carbon metabolism (carbon fixation, glycolysis/gluconeogenesis, tricarboxylic acid cycle (TCA) and oxidative pentose pathway) [20,21]. However, enzymes belonging to those pathways have not been analysed with only few exceptions such as phosphoribulokinase (PRK) and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [22,23]. Like other aquatic photoautotrophs, diatoms can experience very large variations in the availability of key resources such as light, inorganic carbon and nutrients and consequently require efficient mechanisms to regulate their metabolism. Regulation of PRK and GAPDH from the Calvin cycle in many species including diatoms was shown to vary with phylogeny. PRK was redox insensitive in diatoms, while redox regulation of GAPDH was observed in freshwater but not in marine taxa [24]. To allow rational design and engineering of high lipid-producing algae, considerable efforts are thus required to understand the fundamental mechanisms of diatom metabolism. Carbon concentrating mechanisms are still a matter of debate in diatoms, however, it is known that both $CO_2$ and bicarbonate are taken up by these organisms [25]. The relationships between carbon flow and lipid accumulation under different growth conditions are, however, not yet deciphered.

So far, the majority of diatoms studied for lipid contents in connection with biodiesel production were marine species [12] with few exceptions [26]. Recently, four freshwater genera were however analysed with the aim of using natural freshwater diatoms for biofuel feedstock production using waste water [11]. The aim of the present study was to describe the physiology and metabolism of a representative freshwater diatom, *Asterionella formosa*, by measuring the activity of key enzymes (Fig. 1) and TAG contents under various nutrient regimes. Inducing lipid accumulation via nitrate, iron and silicon limitation, was explored as well as adding phytohormones and bicarbonate to try to stimulate growth. Moreover, *A. formosa* is the most abundant diatom within freshwater phytoplankton and it experiences a much greater range of

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