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Overexpression of acetyl-CoA synthetase (ACS) enhances the biosynthesis of neutral lipids and starch in the green microalga *Chlamydomonas reinhardtii*

Rocío Rengel[a], Richard T. Smith[b], Richard P. Haslam[b], Olga Sayanova[b], Marta Vila[a,c], Rosa León[a,*]

[a] Laboratory of Biochemistry, Faculty of Experimental Sciences, Marine International Campus of Excellence (CEIMAR), CEICAMBIO University of Huelva, 21071 Huelva, Spain
[b] Department of Plant Sciences, Rothamsted Research, Harpenden AL5 2JQ, UK
[c] PhycoGenetics SL, C/Joan Miró N°6. 21110 Aljaraque, Huelva, Spain

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ABSTRACT

Genetic engineering can be the solution to achieve the economically feasible production of microalgal based biofuels and other bulk materials. A good number of microalgal species can grow mixotrophically using acetate as carbon source. Moreover, experimental evidence suggests that the biosynthesis of acetyl-CoA could be a limiting step in the complex multifactor-dependent biosynthesis of acylglycerides and point to acetyl-CoA synthetase (ACS) as a key enzyme in the process. In order to test this hypothesis we have engineered the model chlorophyte *Chlamydomonas reinhardtii* to overexpress the endogenous chloroplastic acetyl-CoA synthetase, ACS2. Expression of the *ACS2* encoding gene under the control of the strong constitutive *RBCS2* promoter in nitrogen-replete cultures resulted in a 2-fold increase in starch content and 60% higher acyl-CoA pool compared to the parental line. Under nitrogen deprivation, the *Cr-acs2* transformant shows 6-fold higher levels of *ACS2* transcript and a 2.4-fold higher accumulation of triacylglycerol (TAG) than the untransformed control. Analysis of lipid species and fatty acid profiles in the *Cr-acs2* transformant revealed a higher content than the parental strain in the major glycolipids and suggests that the enhanced synthesis of triacylglycerol in the transformant is not achieved at the expense of membrane lipids, but is due to an increase in the carbon flux towards the synthesis of acetyl-CoA in the chloroplast. These data demonstrate the potential of engineering the chloroplastic ACS to increase the carbon flux towards the synthesis of fatty acids as an alternative strategy to enhance the biosynthesis of lipids in microalgae.

1. Introduction

In the last decades there has been an increasing interest in exploiting microalgae for the production of biofuel precursors, such as triacylglycerol (TAG) and starch, which can be transformed into biodiesel and bioethanol, respectively [1–5]. However, until now the commercially viable production of these compounds has been restricted by the high cost of producing algal biomass at large scale and by the fact that these compounds are usually accumulated under stress conditions, such as nitrogen starvation, which limits algal growth and therefore reduces their overall yield [6].

Genetic engineering of microalgae can provide a solution to increase strain productivity and facilitate the development of the economically feasible production of microalgal based biofuels and other bulk materials [7–11]. The first attempt to engineer TAG biosynthetic pathways in microalgae was the pioneering work of Dunahay and co-workers, carried out in the 1990's within the Aquatic species program (ASP) of the US Department of Energy, to increase the TAG productivity in the diatom *Cyclotella cryptica* by overexpressing the native acetyl-CoA carboxylase (ACCase) [12], which catalyzes the first committal step in fatty acid biosynthesis and is considered a limiting step in lipid biosynthesis; however, the 2-fold increase observed for ACCase activity in the transformants did not lead to an increase in lipid content [13]. Since then, extensive research has been done to increase the content of TAG in plants [14] and microalgae [8], or to modify their fatty acids profiles, enriching the presence of acyl groups that better conform the need of certain nutritional or industrial applications [15,16].

Attempts to engineer lipid production include overexpression of enzymes involved in the biosynthesis of fatty acids [17,73] and/or TAG assembly [18–21] as well as blocking competitive pathways, such starch biosynthesis [22] or catabolism of lipids [23]. Several approaches have also focused on using the transcription factors, which

* Corresponding author at: Lab. Bioquímica y Biología Molecular, Facultad de Ciencias Experimentales, Universidad de Huelva, Avda. Fuerzas Armadas s/n., 21071 Huelva, Spain.
E-mail address: rleon@uhu.es (R. León).

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