Renewable Energy 129 (2018) 347–356

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Evaluation of transcription profile of acetyl-CoA carboxylase (ACCase) and acyl-ACP synthetase (AAS) to reveal their roles in induced lipid accumulation of *Synechococcus* sp. HS01

Sima Modiri [a], Hossein Shahbani Zahiri [a], Hojatollah Vali [b], Kambiz Akbari Noghabi [a, *]

[a] Department of Energy and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), P. O. Box 14155-6343, Tehran, Iran

[b] Facility for Electron Microscopy Research, McGill University, 3640 Street, Montreal, Quebec H3A 0C7 Canada

ARTICLE INFO

Article history:
Received 1 September 2017
Received in revised form
29 March 2018
Accepted 1 June 2018
Available online 2 June 2018

Keywords:
Synechococcus sp. HS01
Ostrich oil
Lipid content
Mixotrophic cultivation
Transcription analysis
De novo fatty-acid synthesis

ABSTRACT

It was earlier shown that the mixotrophic cultivation of *Synechococcus* sp. HS01 in ostrich oil-containing BG11 medium leads to an up to 2.82- fold increase in lipid productivity compared with autotrophic condition. A follow-up investigation was carried out to divulge whether conditions that would increase the lipid content in *Synechococcus* sp. HS01, bringing about constantly more pool towards the main route for fatty acids synthesis. To this end, the expression patterns of two genes including *acc* as the main enzyme involved in fatty acid synthesis pathway, converting Acetyl-CoA to Malonyl-CoA and *aas* gene which convert free fatty acids to active Acyl carrier protein (ACP) (through esterification) were carefully analyzed under different culture conditions.

While there is a direct association between the expression of *acc* gene and changes in lipid content of glucose and acetate-grown cultures, no direct relationship exists between the expression of this gene in ostrich oil-grown culture and nitrogen-deficient conditions. Reduced *acc* gene expression and increased expression of *aas* gene in conjunction with increased lipid contents in ostrich oil-grown cultures indicated that exogenous fatty acids can be applied directly to the intracellular lipid synthesis and a possible strategy for achieving greater amount of lipids produced by cyanobacteria.

1. Introduction

Human insatiable consumption of fossil fuels has led to the serious environmental pollution around the world. The bioenergy production is taken into consideration because of increased concerns about petroleum resource constraints and swift rise of atmospheric CO_2. Therefore, finding adequate sources of clean energy for the future is one of the challenges of society faces today [1]. In this connection, sustainable energy production from algae or cyanobacterial lipids is a perfect solution to replace fossil fuels due to their higher photosynthetic efficiency and potential of lipid production compared to crop plants [2,3]. There are many economic challenges that should be resolved before industrialization of biofuels from microalgae. One of these challenges is the lack of microalgal strains having high lipid content and high growth rate [4]. Another challenge is that, although mixotrophic microalgae cultivation can increase production of biomass and lipids compared with autotrophic culture, the cost of organic carbon substrate contains 80% of the total production expenses [5]. Hence, the use of inexpensive and low-cost carbon sources such as crude glycerol (from biodiesel production), acetate (from anaerobic digestion), and carbohydrates (from agricultural and industrial wastes) through mixotrophic cultivation is more favorable for commercial applications [5].

Metabolic engineering strategies are employed as a solution in the creation of producer strains suitable for biofuels production, leading the metabolic flux toward higher synthesis of more effective intermediates involved in lipid accumulation [6]. Consequently, fundamental studies is required to improve our understanding of the genetic and physiology of these types of microorganisms, particularly in relation to the metabolism of fatty acids [4]. In cyanobacteria, fatty acids are synthesized by a type II fatty acid synthase (FAS) complex. Fatty acid synthesis (FAS) type II provides fatty acid substrates for membrane lipids [7].

* Corresponding author.
E-mail address: Akbari@nigeb.ac.ir (K.A. Noghabi).

https://doi.org/10.1016/j.renene.2018.06.002
0960-1481/

Phosphoenolpyruvate (PEP) is commonly used in fatty acid and protein biosynthesis as a substrate (Fig. 1). Accordingly, the regulation of the fatty acid biosynthesis depends on the relative activity of Acetyl-CoA carboxylase (ACCase) and phosphoenolpyruvate carboxylase (PEPC) enzymes. Once phosphoenolpyruvate (PEP) is being converted into oxaloacetate (by phosphoenolpyruvate carboxylase (PEPC) enzyme), it can be incorporated into the protein synthesis pathway. However, when it is converted into the acetyl-CoA in a two-step reaction (by pyruvate kinase and pyruvate dehydrogenase), it is incorporated into fatty acid synthesis pathway.

Acetyl coenzyme A (acetyl-CoA) is converted to malonyl-CoA (by acetyl-CoA carboxylase) which is the main precursor for fatty acid synthesis. In many bacteria, this substrate catalyzed by type II fatty acid synthase (FAS II) in five reactions. In addition, pyruvate can be directly converted into alanine in the protein metabolism [8,9]. Acetyl-CoA carboxylase (ACC) which catalyzes the conversion of Acetyl-CoA to malonyl-CoA is a multi-subunit enzyme, acting as a key enzyme in fatty acid biosynthesis. This reaction is also known as a rate-limiting step for the biosynthesis of fatty acids [10].

Aas gene encoding proteins as acyl-acyl carrier protein synthetase (AAS) catalyze the ATP-dependent conversion of free fatty acids to acyl carrier protein (ACP) through esterification [11]. Free fatty acids (FFAs) formed by membrane-acting lipolytic enzymes can also be converted to active acyl-ACP by acyl-ACP synthetase (AAS). It has previously been shown that strategies for fatty acid feeding of cyanobacteria has led to the contribution of these fatty acids in the synthesis of membrane lipids [12]. In addition, complex lipids such as phosphatidylglycerol (PG) may also be efficiently taken up by cyanobacteria [13]. Activation of fatty acids is a key step in the metabolism of fatty acids. In cyanobacteria, this activation is performed by acyl-ACP synthetase (AAS), enabling fatty acid recycling in these organisms [12]. Previous studies in our lab showed that the mixotrophic cultivation of *Synechococcus* sp. HS01 in BG11 medium supplemented with glucose, ostrich oil, as well as N-deprivation can lead to an increase in lipid productivity. The ability of cyanobacteria to grow in mixotrophic conditions using crude oil (as carbon source) has earlier been reported [14]. We also showed that the heterotrophic growth of *Synechococcus* sp. HS01 using ostrich oil leads to a 2.82-fold increase in lipid productivity compared to autotrophic growth conditions [15] The analysis of the fatty acid composition of *Synechococcus* sp. HS01 grown in these media by gas chromatography (GC) showed that this strain could be a promising feedstock for biofuel production, especially when cultured in the presence of ostrich oil [15]. In the present study,

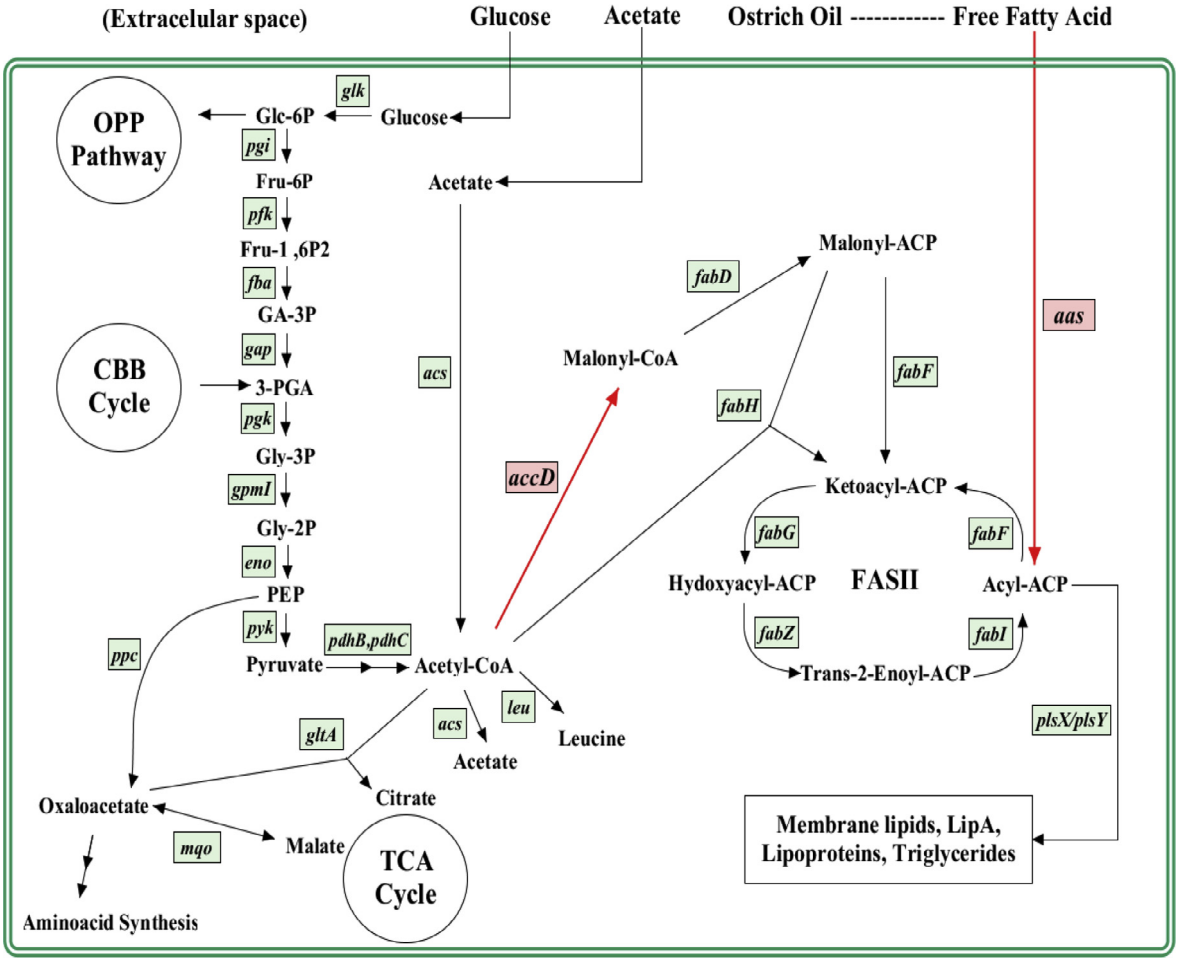

Fig. 1. Schematic diagram of fatty acid biosynthesis and some of the competing pathways in *Synechococcus* spp. The abbreviations for the genes encoding metabolic enzymes included are as follows: glk; glucokinase, pgi; glucose-6-phosphate isomerase, pfk; 6-phosphofructokinase 1, fba; fructose-bisphosphate aldolase, gap; glyceraldehyde-3-phosphate dehydrogenase, pgk; phosphoglycerate kinase, gpmI; 2,3-bisphosphoglycerate-independent phosphoglycerate, eno; enolase, pyk; pyruvate kinase, ppc; phosphoenolpyruvate carboxylase, pdhB; pyruvate dehydrogenase E1 component beta subunit, pdhC; pyruvate dehydrogenase E2 component, gltA; citrate synthase, mqo; malate dehydrogenase (quinone), acs; acetyl-CoA synthetase, leuA; 2-isopropylmalate synthase, accD; acetyl-CoA carboxylase, fabD; [acyl-carrier-protein] S malonyltransferase, fabH; 3-oxoacyl-[acyl-carrier-protein] synthase III, fabF; 3-oxoacyl-[acyl-carrier-protein] synthase II, fabG; 3-oxoacyl-[acyl-carrier protein] reductase, fabZ; 3 hydroxyacyl-[acyl-carrier-protein] dehydratase, fabI; enoyl-[acyl-carrier protein] reductase I, aas; acyl-ACP synthetase, plsX; glycerol-3-phosphate acyltransferase, plsY; glycerol-3-phosphate acyltransferase.