Bioresource Technology 259 (2018) 46–53

Contents lists available at ScienceDirect

Bioresource Technology

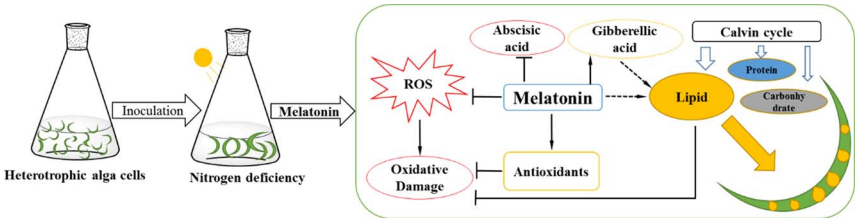
journal homepage: www.elsevier.com/locate/biortech

Melatonin enhances lipid production in *Monoraphidium* sp. QLY-1 under nitrogen deficiency conditions via a multi-level mechanism

Yongteng Zhao[a], Dafei Li[a,1], Jun-Wei Xu[a], Peng Zhao[a], Tao Li[a], Huixian Ma[b], Xuya Yu[a,*]

[a] Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China
[b] School of Foreign Languages, Kunming University, Kunming 650200, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microalgae
Lipid
Melatonin
Nitrogen deficiency
Antioxidant systems
Signalling molecules

ABSTRACT

In this study, melatonin (MT) promoted lipid accumulation in *Monoraphidium* sp. QLY-1 under nitrogen deficiency conditions. The lipid accumulation increased 1.22- and 1.36-fold compared with a nitrogen-starved medium and a normal BG-11 medium, respectively. The maximum lipid content was 51.38%. The reactive oxygen species (ROS) level in the presence of melatonin was lower than that in the control group, likely because of the high antioxidant activities. The application of melatonin upregulated the gibberellin acid (GA) production and *rbcL* and *accD* expression levels but downregulated the abscisic acid (ABA) content and *pepc* expression levels. These findings demonstrated that exogenous melatonin could further improve the lipid production in *Monoraphidium* sp. QLY-1 by regulating antioxidant systems, signalling molecules, and lipid biosynthesis-related gene expression under nitrogen deficiency conditions.

1. Introduction

Fossil fuels are limited non-renewable resources that will run out in a few decades. This phenomenon motivates many researchers to explore new renewable energy sources that could replace fossil fuels. Biodiesel is considered one of the best candidates for this purpose (Mata et al., 2010; Abomohra et al., 2016). The use of oleaginous microalgae as feedstock for biofuel production is also a prospective alternative to terrestrial plant utilization (Hu et al., 2008; Markou and Nerantzis, 2013; Ho et al., 2014).

Improvements in microalgal lipid contents have further increased the use of microalgae as a sustainable feedstock for biodiesel production. The lipid content of microalgae is influenced by some factors (Singh et al., 2016), among which nitrogen starvation is an effective approach for enhancing lipid accumulation in various microalgal species (Pancha et al., 2014; Li et al., 2014a). Stress also increases the microalgae lipid content but radically decreases the biomass concentration (Anand and Arumugam, 2015). Biofuel production requires an enormous amount of biomass for oil extraction. This limitation can be overcome by a two-step strategy comprising heterotrophic cultivation and a photochemical modulator induction (Zhao et al., 2016). High-density heterotrophic algal cells are diluted and transferred to a

* Corresponding author.
E-mail address: xuya_yu@163.com (X. Yu).
[1] The author contributed equally to this work.

https://doi.org/10.1016/j.biortech.2018.03.014
Received 8 February 2018; Received in revised form 28 February 2018; Accepted 1 March 2018

light environment, and chemicals are added for efficient lipid induction in microalgae (Che et al., 2016; Cheng et al., 2017; Li et al., 2017).

Phytohormones and analogues can regulate multiple aspects of microalgae metabolism (Yu et al., 2015; Borowitzka, 2016). Plant hormones are metabolism inducers or enhancers that directly moderate intracellular metabolism and promote the growth and production of lipids in microalgae (Lu and Xu, 2015; Che et al., 2017; Parsaeimehr et al., 2017; Liu et al., 2017). Few studies have reported the application of plant hormones, particularly melatonin, to enhance microalgal biomass and metabolite accumulation (Tal et al., 2015; Li et al., 2017). Melatonin, a plant growth regulator that is involved in the responses to several environmental stress factors (Arnao and Hernández-Ruiz, 2014; Zhang et al., 2015; Reiter et al., 2015; Arnao and Hernández-Ruiz, 2015), belongs to the antioxidant class of plant hormones that promote lipid production, as indicated in our previous study (Li et al., 2017), which mainly focused on plant hormone functions associated with algal growth promotion and lipid induction under optimized growth conditions (Li et al., 2015; Parsaeimehr et al., 2017). However, the effects of phytohormones on microalgae under nitrogen depletion conditions are poorly understood (Renuka et al., 2017; Yu et al., 2018), and the effects of melatonin on algal characteristics under nitrogen starvation remain largely unknown.

Redox reactions of reactive oxygen species (ROS) with cellular lipids, proteins, and DNA result in toxicity due to oxidative stress conditions (Mallick and Mohn, 2000). The oxidative stress caused by high light, high salinity, and nitrogen starvation conditions is associated with lipid biosynthesis in microalgae (Li et al., 2017; Chokshi et al., 2017a,b). Melatonin can act directly as an effective antioxidant that decreases ROS levels and protects plants from oxidative stress (Galano et al., 2011; Zhang et al., 2014; Shi et al., 2015a). Therefore, the cellular crosstalk of ROS-scavenging antioxidants may be induced by melatonin addition in algae.

Melatonin is also involved in signal transduction pathways, including pathways that use auxin (IAA), ethene (ETH), salicylic acid (SA), nitric oxide (NO), and specifically, gibberellic acid (GA) and abscisic acid (ABA), which form a complicated signalling network related to growth, development, and abiotic stress tolerance in plants (Zhang et al., 2014; Li et al., 2014b). Although the basic physiological activities of various phytohormones are well described for higher plants treated with melatonin, their role in microalgae, which is chiefly a role in abiotic stress, and their response to oxidative stress are rarely studied. Furthermore, melatonin plays a critical role in plant signal transduction. However, the purpose of extraneous melatonin in the molecular mechanism of lipid biosynthesis in green microalgae under nitrogen deficiency conditions has not been fully described.

This study aims to analyse the melatonin-induced physiological and biochemical changes in the freshwater microalga *Monoraphidium* sp. QLY-1 under nitrogen starvation. Biomass, lipid, carbohydrate, and protein contents were investigated. The responses of stress biomarkers, such as ROS, malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ABA and GA, were evaluated to study the role of oxidative stress in the lipid accumulation in QLY-1 through nitrogen deficiency integration and melatonin addition. The expression levels of key genes involved in lipid biosynthesis were also analysed. Simultaneous melatonin application and nitrogen starvation under autotrophic conditions provides a novel protocol for microalga-based oil accumulation. The objective is to increase the knowledge regarding the mechanisms of melatonin in increasing microalgal tolerance to ensuing nitrogen deficiency conditions.

2. Materials and methods

2.1. Microalgal strain and culture conditions

Monoraphidium sp. QLY-1 was cultivated in BG-11 medium (Zhao et al., 2016). The seed cells were cultured in flasks under heterotrophic conditions. The heterotrophic algal cells were collected and re-suspended in fresh BG-11 medium under autotrophic conditions. Then, 1 μM melatonin was added for lipid induction; microalgae under nitrogen deficiency conditions without melatonin and normal BG-11 served as the controls. Each group was evaluated independently in triplicate.

2.2. Determination of biomass and lipid contents

Microalgal cells were collected through centrifugation. The fresh algal cell content was estimated after freeze-drying, followed by weighing. Total lipids were extracted from algal powder using a methanol:chloroform solution (1:2, *v*:*v*) (Bligh and Dyer, 1959), as described in our previous study (Li et al., 2017). The total lipids were further fractioned by a column (25 mm × 550 mm) with silicic acid (30 g) using chloroform to collect neutral lipids (NL), acetone to collect glycolipids (GL), and methanol to collect phospholipids (PL) (Zhao et al., 2016).

2.3. Carbohydrate and protein analysis

Lyophilized algal powders from the melatonin-treated cultures and the control cultures were used for the analysis of the total carbohydrate content; glucose was used to prepare a standard curve for analysis of the total carbohydrate content (Jia et al., 2015). Total protein was extracted and measured as previously described with bovine serum albumin (BSA) as a standard (Berges et al., 1993).

2.4. ROS, lipid peroxidation and antioxidant enzyme assays

To estimate the oxidative stress of cells subjected to melatonin induction under nitrogen deficiency conditions, the ROS levels were monitored using 2′,7′-dichlorodihydrofluorescein diacetate (Beyotime, China) as a probe, as previously described (Li et al., 2017). Lipid peroxidation was estimated by measuring the production of MDA with a lipid peroxidation MDA assay kit (Beyotime). The activity of SOD was determined using a SOD assay kit (Beyotime) with measurements performed at 450 nm. POD activities were determined using colorimetric assay kits (Beyotime) based on the absorbance at 430 nm. CAT activity was assayed as previously described (Liu et al., 2013).

2.5. Endogenous ABA and GA levels

An ELISA kit for the plant hormones abscisic acid and gibberellic acid (Enzyme-linked Biotechnology, Shanghai, China) was used to quantify the endogenous ABA and GA levels according to the manufacturer's instructions. The kit assay to determine the plant ABA or GA level in the sample used purified plant ABA or GA antibody to coat microtiter plate wells; the solid-phase antibody was synthesized; and ABA was added to the wells. The antibody was combined with horseradish peroxidase (HRP) to produce an antibody-antigen-enzyme-antibody complex. After complete washing, tetramethylbenzidine (TMB) substrate solution was added. The TMB substrate becomes blue when HRP enzyme performs catalysis, and the reaction was terminated by adding a sulfuric acid solution. The colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of ABA or GA in the samples was then determined by comparing the OD of the samples to the following standard curves:

$$Y_1 = 232.0836X_1 - 13.0438,\ R^2 = 0.9991;$$

$$Y_2 = 232.0836X_2 - 13.0438,\ R^2 = 0.9991;$$

where Y_1 and Y_2 represent the concentrations of ABA and GA, respectively, and X represents the absorbance of OD450 nm.