Phytochemistry 139 (2017) 25–32

Contents lists available at ScienceDirect

Phytochemistry

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

UV–C radiation increases sterol production in the microalga *Pavlova lutheri*

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ARTICLE INFO

Article history:
Received 9 January 2017
Received in revised form
5 April 2017
Accepted 6 April 2017

Keywords:
Pavlova lutheri
Microalgae
Plant sterols
UV–C radiation
Sterol profiles

ABSTRACT

Plant sterols have become well-known to promote cardiovascular health through the reduction of low density lipoprotein cholesterol in the blood. Plant sterols also have anti-inflammatory, anti-cancer, anti-oxidative and anti-atherogenicity activities. Microalgae have the potential to become a useful alternative source of plant sterols with several species reported to have higher concentrations than current commercial ones. In order to increase phytosterol production and optimise culture conditions, the high sterol producer *Pavlova lutheri* was treated in different dosages (50–250 mJ m^{-2}) of UV–C radiation and several concentrations (1–500 μmol/L) of hydrogen peroxide (H_2O_2) and the sterol contents were quantified for two days after the treatments. The contents of malondialdehyde (MDA) superoxide dismutase (SOD) as indications of cell membrane damage by lipid peroxidation and repair of oxidative stress, respectively, were measured. Higher activities of SOD and MDA were observed in the treated biomass when compared to the controls. Total sterols increased in *P. lutheri* due to UV–C radiation (at 100 mJ m^{-2}) but not in response to H_2O_2 treatment. Among the nineteen sterol compounds identified in *P. lutheri*, poriferasterol, epicampesterol, methylergostenol, fungisterol, dihydrochondrillasterol, and chondrillasterol increased due to UV–C radiation. Therefore, UV–C radiation can be a useful tool to boost industrial phytosterol production from *P. lutheri*.

1. Introduction

Sterols are important constituents of eukaryotic cell membranes; together with phospholipids they maintain stability, fluidity and permeability of cell membranes (Dufourc, 2008). Sterols are very diverse in plants and this diversity has been a useful tool for chemotaxonomic and phylogenetic comparisons of various plant species (Francavilla et al., 2010). Plant sterols or phytosterols have become particularly interesting for the biotechnology industry in the last few decades due to the discovery that they can significantly reduce the intestinal absorption of dietary and biliary low density lipoprotein (LDL) cholesterol and help maintain good cardiovascular health (Carmona et al., 2010). Because of such properties, phytosterols are currently used as additives in various products such as milk, margarines and yoghurt (Carmona et al., 2010). Moreover, phytosterols are reported to potentially provide protection against nervous system disorders such as amyotrophic lateral sclerosis, autoimmune encephalomyelitis, or Alzheimer's disease and they may also possess anti-oxidative, anti-inflammatory, anti-atherogenicity and anti-cancer properties (Kim et al., 2008; Breitner and Welsh-Bohmer, 2000). The applications of phytosterols in the industry also include cosmetics (e.g. lipstick and creams), and therapeutic steroids (Fernandes and Cabral, 2007).

Current industrial sources for phytosterols are tall oil and vegetable oils. With an expected growth of 7–9% per year from the current global market base ($300 million), phytosterols represent one of the highly lucrative sectors in the biotechnology industry (Borowitzka, 2013). Among the other various sources of phytosterols, microalgae can be very useful due to the well-known advantages that they are relatively easy to cultivate in any kind of lands (both arable and non-arable) eliminating competition with food production, they can adapt to varying environmental conditions by producing various specialised metabolites, and they can also be used to purify and take up nutrients from wastewater

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http://dx.doi.org/10.1016/j.phytochem.2017.04.002
0031-9422/

(Ahmed et al., 2014). Although many microalgal strains have been studied for distribution of different sterol compounds (Volkman, 2003), the potential for industrial production of phytosterols from these strains has remained mostly unexplored. Recently, *Dunaliella tertiolecta* Butcher and *D. salina* Dunal have been reported as promising microalgal strains for phytosterols due to good quantities (0.9–1.3% dry weight, DW) and *in vivo* immunomodulatory activity (Francavilla et al., 2012). Moreover, the haptophyte microalga *Pavlova lutheri* Droop is found to have high contents of sterols (up to 5.1% DW) requiring further studies to increase its production and also to understand the trigger mechanism of the accumulation of such high quantities (Ahmed et al., 2015a). Among the different factors known to affect sterol production in microalgae are growth conditions such as salinity of the medium (Peeler et al., 1989; Zelazny et al., 1995; Francavilla et al., 2010), renewal rate of semi-continuous cultures (Fabregas et al., 1997), photobioreactor type (Ponis et al., 2006), the stage of growth (Xu et al., 2008), nutrient (e.g. phosphorus, and silicate) content, light, temperature (Piepho et al., 2010, 2012) and age of the cultures (Ahmed et al., 2015a).

Recently, UV–C radiation (100–280 nm) has been reported as a useful tool for inducing lipids especially unsaturated fatty acids (Sharma et al., 2014) and carotenoid production (Ahmed et al., 2015b) in several strains of microalgae. It is well known that band C of the UV radiation does not reach the surface of the Earth; however, this band possesses the highest energy per photon. UV radiation is suggested to cause damage to the thylakoid membranes of the cells leading to the production of reactive oxygen species (ROS; Huang and Cheung, 2011; Sharma et al., 2014). We hypothesize that sterols being an integral part of the membranes and bearing the responsibility of maintain the integrity of the cell membranes would play an active role in repairing the damage caused to the cells by UV radiation. Additionally, the production or addition of hydrogen peroxide (H_2O_2) in the medium is well known to cause oxidative damage to the cells through the production of reactive oxygen species (ROS). This induces the accumulation of antioxidants such as carotenoids (e.g. astaxanthin, lutein) in the cells (Wei et al., 2008; Ip and Chen, 2005). Due to the discovery that sterols could act as antioxidants (Fernandes and Cabral, 2007), it can be hypothesised that the presence of H_2O_2 in the medium would also increase the accumulation of sterols in microalgae. However, to our knowledge, no information is available so far on the effects of the use of UV–C radiation or H_2O_2 treatment on sterol production in any microalgae species.

In this study, we report the effects of UV–C radiation and hydrogen peroxide (H_2O_2) treatment on sterol production in the commercially important microalga *Pavlova lutheri*. We also report the contents of malondialdehyde (MDA), the measure of lipid peroxidation as a result of oxidative stress and the accumulation of the antioxidant enzyme superoxide dismutase (SOD), under UV–C radiation and H_2O_2 treatment in order to understand the level of oxidative stress and antioxidant production in relation to these treatments.

2. Results and discussion

2.1. Contents of SOD and MDA increased after UV–C or H_2O_2 treatments

The contents of MDA indicate damage in the cell membranes due to lipid peroxidation through exposure to oxidative stress (Raposo et al., 2015). SOD detoxifies the effects of oxidative stress produced especially by superoxide radicals by dismutating them into hydrogen peroxide and oxygen. SOD, therefore, prevents hydroxyl radical formation (Cirulis et al., 2013). The contents of MDA and SOD had similar patterns under both UV–C radiation and H_2O_2 treatment. Significant differences were found between the contents of MDA and SOD at different sampling times and at different dosages of UV–C radiation (two-way ANOVA: $p < 0.05$; Supplementary Table 1). The contents of MDA and SOD under UV–C radiation were significantly higher at dosages from 100 to 250 mJ cm^{-2} when compared to controls at all sampling times (Tukey's test: $p < 0.05$; Fig. 1). Although the contents were similar in controls in the 24 h and 48 h samples (MDA: 0.178 ± 0.023 to 0.245 ± 0.024 mmol/10^6 cells; SOD: 0.174 ± 0.003 to 0.211 ± 0.011 U/10^6 cells), they went up in the irradiated biomass at 24 h after the irradiation (MDA: 0.786 ± 0.028 to 4.637 ± 0.08 mmol/10^6 cells; SOD: 0.378 ± 0.003 to 4.74 ± 0.316 U/10^6 cells) and then lowered in all irradiated cultures at 48 h after the radiation (MDA: 0.654 ± 0.025 to 2.923 ± 0.149 mmol/10^6 cells; SOD: 0.275 ± 0.003 to 2.535 ± 0.229 U/10^6 cells; Fig. 1).

Similar to UV–C radiation, significant differences were also found in the contents of both MDA and SOD at different sampling times due to different concentrations of H_2O_2 (two-way ANOVA: $p < 0.05$; Supplementary Table 1). Both MDA and SOD contents peaked at 24 h under H_2O_2 treatment as well (Tukey's test: $p < 0.05$; Fig. 2). The contents of MDA in the treated cultures ranged from 0.323 ± 0.02 U/10^6 cells (1 µmol/L on day 2) to 4.659 ± 0.101 U/10^6 cells (500 µmol/L on day 1). The SOD in the treated cultures ranged from 0.257 ± 0.008 U/10^6 cells (1 µmol/L on day 2) to 5.935 ± 0.358 U/10^6 cells (500 µmol/L on day 1; Fig. 2).

Similar trends in the concentration and time-dependant increase of SOD production have already been reported in *Scenedesmus vacuolatus, Scenedesmus* sp., *Pavlova viridis, Chlorococum* sp.

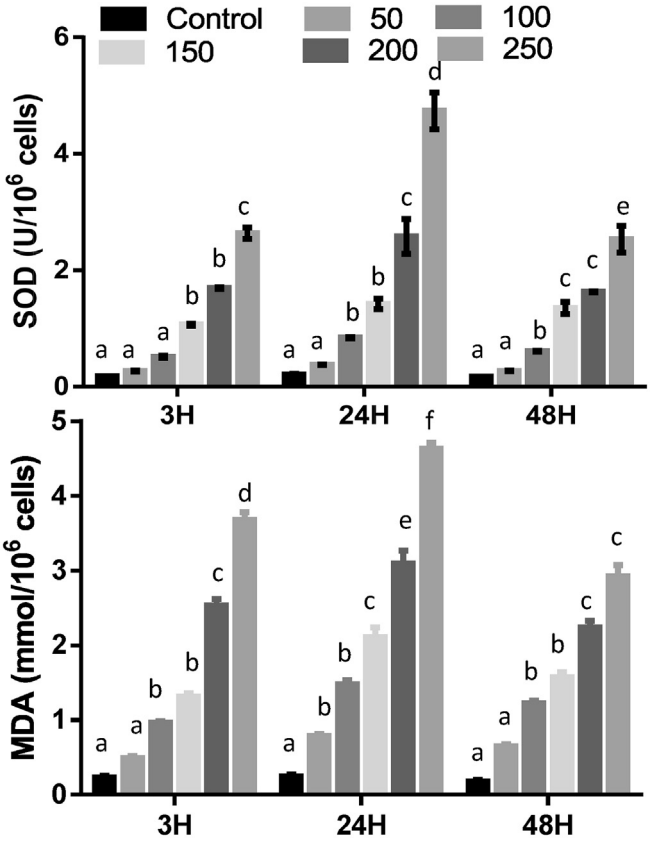

Fig. 1. Contents of superoxide dismutase (SOD) and malondialdehyde (MDA) at different dosages of UV–C radiation (mJ cm^{-2}) after 3, 24 and 48 h of treatment. Shown are mean values and SEs from three separately-grown cultures, each. Different letters indicate statistically significant differences (Tukey's test; $p < 0.05$).