Biochemical Systematics and Ecology 64 (2016) 131–135

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Diel variation of sesquiterpene elatol production: a chemical defense mechanism of the red seaweed *Laurencia dendroidea*

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ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form 3 December 2015
Accepted 11 December 2015
Available online 29 December 2015

Keywords:
Laurencia dendroidea
Clone
Secondary metabolite
Diel variation
Elatol

ABSTRACT

Variability in the production of secondary metabolites by seaweeds is commonly measured under natural conditions and is usually considered a response to environmental factors. However, it is not well known if levels of secondary metabolites can vary due to internal regulation of physiological processes. We measured the diel levels of elatol - the major defensive compound of *Laurencia dendroidea* - in correlation with the activity of photosystem II (Φ_{PSII}) in clones of this seaweed cultivated under constant conditions of temperature, salinity, nutrient availability and irradiance. Our results showed temporal variation in amounts of elatol, which peaked at the beginning of the light and dark periods. The Φ_{PSII} during the light phase, as measured by chlorophyll fluorescence imaging, was higher when the elatol concentration was lower. We conclude that under constant conditions, i.e. without the influence of natural habitat and genetic variability, there is diel variation in elatol production that is inversely correlated with Φ_{PSII}. We suggest that both metabolic activity of secondary metabolite production and photosynthetic activity are under an endogenous control in this red seaweed. We discuss the possible adaptive responses of this phenomenon to environmental pressures.

1. Introduction

Several seaweeds produce structurally diversified secondary metabolites that have important ecological roles as defense and/or signal compounds (Amsler, 2008). Red seaweed species are the richest in terms of halogenated secondary metabolites, such as those observed in *Laurencia* species (Ceramiales, Rhodophyta) that are specialized in the production of bromide and chloride terpenoid compounds (e.g. Blunt et al., 2013).

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http://dx.doi.org/10.1016/j.bse.2015.12.001
0305-1978/

These terpenoids are characteristic components that occur in relatively low concentrations in seaweeds, ranging from 0.2% to 2.0% of algal dry mass (Paul and Fenical, 1986, 1987), but their levels are not constant. The concentrations of these chemicals can vary in different parts of the cell (Salgado et al., 2008), and at intra-thallus (Sudatti et al., 2008), intra-populational (Sudatti et al., 2006) and among population (Oliveira et al., 2013) levels. This variability arises due to distinct abiotic or biotic environmental conditions, such as herbivore pressure or induction (e.g. Flöthe and Molis, 2013), nutrient load (Van Alstyne and Pelletreau, 2000), depth (Gerwick et al., 1985), irradiance (Pavia et al., 1997), and temperature and salinity (Sudatti et al., 2011).

Variation in secondary metabolite concentrations is of significant ecological importance for the establishment of biological interactions, with consequences for population and community structure (Hay, 2009). The sesquiterpene elatol found in *Laurencia dendroidea* J. Agardh (previously named *Laurencia obtusa*; see Sudatti et al., 2011) is a broad spectrum feeding deterrent against sea urchins (Pereira et al., 2003), reef fishes (Paul et al., 1988), snails (Granado and Caballero, 1995) and fouling organisms (Da Gama et al., 2002). Due to these multiple ecological roles, it can be assumed that variability in concentrations of this chemical are important in the establishment of interactions of *Laurencia* species with consumers, competitors and even epibionts.

Although several studies have revealed information about factors that influence the levels of secondary metabolites in seaweeds, there is a notable gap in terms of diel dynamics. Here, we present the first assessment of the amounts of the sesquiterpene elatol produced during a 24 h cycle in cultivated clones of *Laurencia dendroidea*.

2. Materials and methods

2.1. Clonal culture conditions

Specimens of *L. dendroidea* used in this study were collected at Cabo Frio Island, Rio de Janeiro State, Brazil and transported to the laboratory to develop a unialgal culture (see Sudatti et al., 2011). Voucher specimens (SP 399789) have been housed at Instituto de Botânica Herbarium, São Paulo State, Brazil.

The biomass used to quantify sesquiterpene elatol during 24 h cycles was propagated from only one genotype. Non-fertile clonal replicates ($n = 40$) weighing 15–30 mg (wet mass) were incubated in sterile seawater (80 ml) enriched with 50% von Stosch solution at 22 ± 2 °C, salinity 32 ± 1% and irradiance 60–80 μmol photons/m^2/s (provided by cool-white fluorescent lamps with a 14:10 h light:dark cycle), without aeration. Lights turned on and off at 06:00 and 20:00 h, respectively.

2.2. Temporal variation of elatol in Laurencia dendroidea

From 08:30 h, four clones (replicates) of *L. dendroidea* were removed from the culture every 3 h over a period of 27 h and elatol concentrations in crude extracts were quantified by gas chromatography equipped with an electron-capture detector (GC-ECD). Differences between mean levels of elatol per sampling period were evaluated by ANOVA/Tukey test (Statistica 8.0 software).

2.3. Chemical procedures

Crude extracts in 20 ml hexane were obtained from each individual clone of *L. dendroidea* at intervals of one week, repeated three times. Elatol quantification was performed in a GC-ECD by the external standardization method described by Sudatti et al. (2006), with a few modifications. The GC-ECD (Chrompack, Netherlands) was fitted with a RTX-5 capillary column (30 m × 0.25 mm, 5% phenyl, 95% dimethylpolysiloxane; Restek, USA). The oven temperature program was as follows: 80 °C (1 min), 10 °C/min to 250 °C (16 min), then −15 °C/min to 80 °C. Nitrogen (99.999%, White Martins) was used as carrier (28 cm/s), makeup (35 ml/s) and purge (15 ml/s). The detector temperature was set at 320 °C and the injection pressure was 120 kPa. On column injections were carried out (0.5 μl). Purified elatol was used as an external standard and quantifications were made through analytical curves ($R^2 = 0.99$) by plotting the injected elatol mass against the peak area calculated based on six different concentrations of standard solutions in hexane. The elatol retention time was verified at 17.8 min. The limits of detection and quantification were 0.048 and 0.112 μg/ml, respectively. Isolation of standard elatol was carried out using precoated TLC (thin layer chromatography) plates, and identified by TLC (Merck Al TLC 20 × 20 cm silica gel $60F_{254}$) and ^{1}H NMR (nuclear magnetic resonance), and compared with the literature (König and Wright, 1997). Recently, this molecule was described as an enantiomer, (−)-elatol (Machado et al., 2011).

2.4. Chlorophyll fluorescence

Clones of *L. dendroidea* were transferred to the laboratory and kept under the same conditions as previously described for three days prior to measuring chlorophyll fluorescence. Six individual replicates were placed in a 24-well plate (3 ml per well) filled with sterile seawater, and were then simultaneously measured by a chlorophyll fluorescence image system.

Determination of the quantum yield of photosystem II (Φ_{PSII}) was undertaken using an imaging system developed at NUPEM/UFRJ that uses a measuring method similar to that described in Siebke and Weiss (1995a, b), Rascher et al. (2001), and Duarte and Lüttge (2005, 2007). The photosynthetic and excitation lights were provided by four arrays of 36 blue light-