Antioxidant response of the brown algae *Dictyota dichotoma* epiphytized by the invasive red macroalga *Lophocladia lallemandii*

Silvia Tejada1, Antoni Sureda2*

1Experimental Laboratory, Research Unit, Son Llàtzer Hospital, IUNICS, Ctra. Manacor km 4, E-07198 Palma de Mallorca, Balearic Islands, Spain.

2Research Group on Community Nutrition and Oxidative Stress, University of Balearic Islands, E–07122 Palma de Mallorca, Balearic Islands, Spain, and CIBERobn CB12/03/30038, Instituto de Salud Carlos III (ISICIII), Spain

**Objective:** To evaluate the response of the brown alga *Dictyota dichotoma* (*D. dichotoma*) epiphytized by the red alga *Lophocladia lallemandii* in Mallorca coastal waters (Balearic Islands) by means of biomarker measures.

**Methods:** Samples of epiphytized and non-epiphytized *D. dichotoma* were collected in Cala Morlanda (East Mallorca, Balearic Islands). Markers of lipid peroxidation and activities of antioxidant enzymes were measured in *D. dichotoma*.

**Results:** Lipid peroxidation measured as malondialdehyde and all the antioxidant activities measured were significantly higher in the epiphytized brown algae when compared with the control algae.

**Conclusions:** In conclusion, the invasive algae *Lophocladia lallemandii* seems to produce a more oxidized status in the epiphytized *D. dichotoma* and cellular damage that could induce increased mortality.

**PEER REVIEW**

**Peer reviewer**
Dr. Comte Katia, MNHN, UMR7245, 12, rue BUFFON–CP90, 75231 Paris Cedex 05 France.
Fax: +33 0 140 793594
E-mail: kcomte@mnhn.fr

**Comments**
The authors have shown in the present work, a significant lipid peroxidation (MDA) and scavenging enzyme activity (SOD, CAT, GRD, GPX) in the brown algae when exposed to the epiphytic algae, in comparison to the control algae (i.e., without epiphytes). This tends to suggest an oxidative stress response of the host–plant due to the presence of algal invaders.

Details on Page 365

**ABSTRACT**

**Objective:** To evaluate the response of the brown alga *Dictyota dichotoma* (*D. dichotoma*) epiphytized by the red alga *Lophocladia lallemandii* in Mallorca coastal waters (Balearic Islands) by means of biomarker measures.

**Methods:** Samples of epiphytized and non–epiphytized *D. dichotoma* were collected in Cala Morlanda (East Mallorca, Balearic Islands). Markers of lipid peroxidation and activities of antioxidant enzymes were measured in *D. dichotoma*.

**Results:** Lipid peroxidation measured as malondialdehyde and all the antioxidant activities measured were significantly higher in the epiphytized brown algae when compared with the control algae.

**Conclusions:** In conclusion, the invasive algae *Lophocladia lallemandii* seems to produce a more oxidized status in the epiphytized *D. dichotoma* and cellular damage that could induce increased mortality.

**KEYWORDS**
Mediterranean Sea, Alien species, Oxidative stress, Antioxidants

---

1. Introduction

Invasive macroalgae are a current problem around all coastal waters in the Western Mediterranean. The red alga *Lophocladia lallemandii* (Montagne) F. Schmitz (*L. lallemandii*) is considered as an alien species in the Mediterranean Sea and it was probably introduced via the Suez Channel1. This alga is very aggressive invasive species and it settles over all types of substrates such as bare bedrocks, macroalgae on rocky bottoms, *Posidonia oceanica* seagrass meadows, and coralligenous communities.

During normal cellular activities, the organelles (chloroplast, mitochondrion, peroxisome) suffer various processes inside the cells that produce reactive oxygen species (ROS), since they present a highly oxidizing metabolic activity or due to the photosynthetic electron
transport chains[2]. Excess of ROS leads to the oxidation of biological macromolecules such as nucleic acids, proteins, carbohydrates and lipids which results in oxidative stress and cellular damage. Cells contain a complex network of antioxidant defences that scavenge or prevent the generation of ROS, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductasa (GRD). In order to protect the cell from the oxidative damage, the free radical O$_2^•$ is metabolised to hydrogen peroxide by SOD, then the hydrogen peroxide is decomposed to water and molecular oxygen by GP and CAT preventing the generation of hydroxy radicals, the most reactive species derived from oxygen[3,4]. The epiphytism of L. lallemandii over algae reduces light availability and hampers water movement; these circumstances enhance organic and nutrient enrichment and oxygen consumption resulting in a stressful situation. Moreover, L. lallemandii are a source of lophocladines, alkaloid molecules with cytotoxic effects[5] and consequently, can negatively affect the development of other macroalgae[6]. Dictyota dichotoma (Hudson) J.V. Lamouroux (D. dichotoma) is a brown algae present in all the oceans and in the Mediterranean Sea. In fact, it has been defined as the only cosmopolitan species of the genus by some authors[7]. Moreover, biological studies have shown a significant number of dictyota secondary metabolites to possess cytotoxic, anti-bacterial, ichthyotoxic and anti-feedant activities[8-10]. However, to our knowledge, oxidative stress studies in D. dichotoma are lacking. In accordance, the aim of the present work was to evaluate the antioxidant response of the brown alga D. dichotoma under stress due to the epiphytism of L. lallemandii by means of the existence of oxidative lipid damage and the response of the CAT, SOD, GPX and GR activities.

2. Materials and methods

2.1. Algae sampling and processing

All samples were carried out in Cala Morlanda (Mallorca, Balearic Islands, western Mediterranean; Figure 1; between 39°33’26.11″N, 3° 22’9.77″E and 39°33’26.68″N, 3°22’14.04″E) the same day during summer time (August 2013). Twelve individuals of D. dichotoma (Hudson) Lamouroux were collected the same day in the following conditions: (1) D. dichotoma epiphytized by L. lallemandii (n=6) and (2) D. dichotoma not epiphytized by L. lallemandii (n=6). All D. dichotoma samples were collected at a similar depth (1-2 m) by expert apnea divers. After collection, algae were transported to the laboratory in a cooler at 5-7 °C. Once in the laboratory, algae were carefully separated from epiphytes and the dead matrix by scratching its surface, using a dull scalpel, in running water and rinsed several times in distilled water. Then, samples of Dictyota were immediately stored at −75 °C until biochemical analysis.

![Figure 1. Map of the sampling location in Mallorca Island (Cala Morlanda) (www.maps.google.com).](image)

Six replicates of D. dichotoma samples were homogenised with a Potter–Elvehjem glass/Teflon (Anorsa, Barcelona, Spain) homogeniser in five volumes (w/v) of 100 mmol/L Tris–HCl buffer at pH 7.5. Each homogenate was centrifuged at 8,000 r/min at 4 °C for 10 min. After centrifugation, supernatants were collected and immediately used for biochemical assays. All assays were performed in duplicate and results were corrected to the total protein content of the samples (Biorad Protein Assay, Biorad Laboratories, Madrid, Spain), using bovine serum albumin as standard.

2.2. Antioxidant enzyme activities

CAT activity (K/s/mg protein) was measured by the method based on the decomposition of H$_2$O$_2$[11]. SOD (pmol/min/mg protein) activity was determined by the degree of inhibition of the reduction of cytochrome C by the superoxide anion generated by the xanthine oxidase/hypoxanthine system. The activity was recorded at a wavelength of 550 nm[12]. GPX activity (nmol/min/mg protein) was measured using H$_2$O$_2$ as substrate[13]. The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ was indicative of the enzyme activity. GR activity (pmol/min/mg protein) was measured by the rate of conversion of oxidized glutathione to reduced glutathione estimated by monitoring oxidation of NADPH in the assay system at 340 nm[14]. All antioxidant enzyme activities were determined with a ShimadzuUV–2100 spectrophotometer at 25 °C.

2.3. Malondialdehyde (MDA) determination

The concentration of MDA (mmol/mg protein), as a marker of lipid peroxidation, was analysed by a specific colorimetric assay kit for MDA determination (Calbiochem®, San Diego, CA, USA), following the manufacturer’s instructions. Briefly,
samples or standard were placed in glass tubes containing n–methyl–2–phenylindole in acetonitrile:methanol (3:1). HCl was added and samples were incubated for 1 h at 45 °C. Absorbance was measured at 586 nm and the concentration of MDA was calculated using a standard curve of known concentrations.

2.4. Statistical analysis

Statistical analysis was carried out using a statistical package (SPSS® v. 19.0 for Windows®). The homogeneity of the variance was assessed by the Kolmogorov–Smirnov test. Statistical significance of the data was assessed by independent samples t-test. Results were expressed as mean±SEM (Standard Error of the Mean) and P<0.05 was considered statistically significant.

3. Results

There was no presence of the invasive algae L. lallemandii in D. dichotoma collected at the control site. No significant differences were evidenced in the protein content between control samples and the samples in the areas where the epiphytism of L. lallemandii over algae D. dichotoma was present [0.185±0.009] mg/mL in control vs. (0.181±0.006) mg/mL in epiphytized].

Lipid peroxidation was measured by the amount of MDA, as the marker of lipid damage. This measure is shown in Figure 2. MDA values were significantly increased in the epiphytized D. dichotoma samples when compared with the control algae (P<0.05).

4. Discussion

The the epiphytic growth of L. lallemandii over the D. dichotoma algae in Cala Morlanda waters (Mediterranean Sea) could induce an environmental stress situation by altering the adequate oxygenation and reducing the irradiance reaching the algae. No significant differences were evidenced in the protein content between control and epiphytized areas. In consequence, the data describing changes in MDA and antioxidant enzyme activities are not a consequence of a reduction in protein content in D. dichotoma epiphytized by L. lallemandii.

L. lallemandii is a red filamentous alga which usually appears as a mat of red filaments intertwined with themselves or with other algae. The epiphytism of L. lallemandii over D. dichotoma is mainly observed in the summer and autumn due to the tropical affinities of genus; in fact, L. lallemandii better develop with higher summer temperatures[15]. Due to its high invasive potential, L. lallemandii is able to cover most kinds of substrate, such as algae communities, resulting in a reduction of density and growth of these algae that can lead to stressful situation and death for native species[1,16,17].

Cellular antioxidant status is used to evaluate the ability of organisms to resist an environmental stress situation[18]. Lipid peroxidation, measured by the amount of MDA, and the antioxidant enzymes which play an important role in protecting from oxidative damage, are both biomarkers of oxidative stress[19]. D. dichotoma epiphytized by L. lallemandii appeared to undergo an oxidative stress, since a significant increase in MDA concentration was observed. The antioxidant defence system seemed to have been overwhelmed, since the antioxidant enzyme activities were not strong enough to prevent membrane lipid peroxidation.
It has been evidenced that algae in order to defence against herbivore pressure produce higher concentrations of defensive compounds[20]. Moreover, the production of ROS has been shown to have a significant contribution towards the survival of algae against pathogens[21]. It has been suggested that the release of H$_2$O$_2$ may act as a chemical defence against herbivores and epiphytes or as an allochemical in direct competition with other algal species[22]. H$_2$O$_2$ has also been reported to act as cellular messenger for the induction of the antioxidant defence system in response to an oxidative stress situation[23]. In accordance, our group has previously evidenced an increased H$_2$O$_2$ production in epiphyted *Posidonia oceanica* suggesting that oxidative stress is involved in the interaction of the invasive *L. lallemandii* and the seagrass[24].

The increase of antioxidant enzyme activities is related to the higher production of ROS, which will be detoxified as result of the antioxidant reactions. However, in the present study, the MDA level in epiphytized *D. dichotoma* was increased indicating that this species is very susceptible to suffer from oxidative stress induced by *L. lallemandii*. The current results are in accordance with previous studies that reported an increase in antioxidant defences of several organisms affected by *L. lallemandii* epiphytism. The invasion of *Posidonia oceanica* meadows by *L. lallemandii* and the growing of this alga on the endemic bivalve *Pinna nobilis* and on the bryozoan *Reteporella grimaldii* induced oxidative stress in these organisms as evidenced by increased levels of oxidative stress markers and in the antioxidant defences[24–26]. *Caulerpa taxifolia* epiphytized by *L. lallemandii* also responded by increasing the production of the toxic metabolite caulerpenyne and H$_2$O$_2$ and increasing the antioxidant enzymes activities as a defensive mechanism[6]. In another study, sea urchins fed during three months with *L. lallemandii* responded with an increased antioxidant response enough to avoid oxidative damage[27].

In conclusion, the present results reported that the interaction of the native *D. dichotoma* with invasive species of macroalgae such as *L. lallemandii* could alter the normal environmental conditions surrounding the algae. The epiphytism of *L. lallemandii* over *D. dichotoma* constitutes a new impact to the algae, resulting in oxidative stress evidenced with an increased antioxidant enzyme activities and lipid peroxidation that could alter the growth or physiology of the native species. Further studies are necessary to elucidate if this algae interaction would result in a decrease of the *D. dichotoma* abundance.

**Acknowledgements**

This work was supported by the Spanish Ministry of Health and Consumption Affairs with grant No. CIBERObn CB12/03/30038, and Balearic Island Government and FEDER funds with grant No. 23/2012.

**Comments**

**Background**

Interactions between native and introduced algal species represent a threat to biodiversity and ecosystem functioning, especially in enclosed Mediterranean Sea. The red algae *L. lallemandii* is able to cover most kinds of substrates including macroalgae and becoming epiphytic. The invasive species can be a cause for the progressive regression of seagrasses.

**Research frontiers**

This study was carried out to determine some stress responses and negative effect of epiphytic invasive *L. lallemandii* on the native brown algae: *D. dichotoma*, by focusing on ROS scavenging enzymes and lipid peroxidation (in comparison to a no–stressed algae: *i.e.* without epiphytes).

**Related reports**

The invasive *L. lallemandii* is commonly investigated in many ecological studies as it is widespread through tropical and temperate areas in many oceans, leading to a loss of biodiversity. However, not many studies reported their potential negative physiological effects on the plants or algae they invaded.

**Innovations and breakthroughs**

The brown algae, host–plant, was commonly known to produce many secondary metabolites including antibacterial activities. However, no previous work was assessed on the oxidative stress of this brown algae in response to an epiphytic algae invader (*i.e.* biotic interaction).

**Applications**

The invasive species are often reported in the literature as inducing deleterious effects on native species in marine and ecosystems. This study supports these findings by focusing on biomarker (*i.e.* antioxidant enzymes and lipid peroxidation) levels in the detection of increased ROS under oxidative stress conditions.

**Peer review**

The authors have shown in the present work, a significant lipid peroxidation (MDA) and scavenging enzyme activity
(SOD, CAT, GRD, GPX) in the brown algae when exposed to the epiphytic algae, in comparison to the control algae (i.e. without epiphytes). This tends to suggest an oxidative stress response of the host-plant due to the presence of algal invaders.

References


