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Antioxidant response of the bivalve *Pinna nobilis* colonised by invasive red macroalgae *Lophocladia lallemandii*

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ABSTRACT

Invasive species represent a risk to natural ecosystems and a biodiversity hazard. The present work aims to determine the antioxidant enzyme response – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), the phase II detoxifying enzyme – glutathione *S*-transferase (GST) – and markers of oxidative damage – thioredoxin reductase (TR) and malondialdehyde (MDA) – in gills and digestive gland of *Pinna nobilis* and to study the antioxidant response effects in the bivalve colonised by the invasive macroalgae *Lophocladia lallemandii*. Colonised specimens were collected in a control area without *L. lallemandii* and another area completely colonised by *L. lallemandii*. All enzyme activities were found to be present in gills and digestive gland, with some tissue differences. CAT and SOD activities were higher in gills than digestive gland, whereas GST activity and MDA levels were higher in digestive gland. The presence of *L. lallemandii* induced a significant increase in the activities of antioxidant enzymes in both gills and digestive gland, except for CAT activity in gills. GST and TR activities were also increased in both tissues, as well as the MDA concentration. We can conclude that the presence of *L. lallemandii* colonising *P. nobilis* induces a biological stress and oxidative damage to the fan mussel.

1. Introduction

The fan mussel *Pinna nobilis* L. is the largest endemic bivalve in the Mediterranean Sea (Garcia-March et al., 2007). Shell size growth can reach 120 cm (Zavodnik et al., 1991) and *P nobilis* appears in coastal areas between 0.5 and 60 m depths, mostly in soft-sediments overgrown by seagrass meadows of *Posidonia oceanica* or *Cymodocea nodosa* (Zavodnik, 1967; Zavodnik et al., 1991). The population of *P. nobilis* has been greatly reduced during the last decades (Vicente and Moreteau, 1991) as a result of recreational and commercial fishing for food, the use of its shell for decorative purposes, and incidental killing by trawling and anchoring. Nowadays, *P. nobilis* is under strict protection and all forms of deliberate capture or killing them are prohibited (EEC, 1992).

Invasive species can introduce severe perturbations into invaded ecosystems. Such species can decrease the biodiversity and alter the structure and function of ecosystems (Boudouresque and Verlaque, 2002; MacDougall and Turkington, 2005; Mack et al., 2000). The red macroalgae *Lophocladia lallemandii* (Montagne; F. Schmitz) – an alien Mediterranean species introduced through the Suez Canal – is widespread throughout the tropics and subtropics (Boudouresque and Verlaque, 2002; Verlaque and Fritayre, 1994). Specifically, *L. lallemandii* grows over all types of substrates (bare bedrocks, rocky macroalgae bottoms, *P. oceanica* seagrass meadows and over coral communities) affecting the invertebrate community (Ballesteros, 2006; Patzner, 1998). *L. lallemandii* is an aggressive species which is able to colonise *P. oceanica* meadows to such a degree that it completely covers great areas of the seagrass meadows (Ballesteros et al., 2007). *L. lallemandii* displays a particular pattern of invasion in *P. oceanica* meadows: Initially, the algae settle on rhizomes; occasionally, they also settle over old leaves, growing as an epiphyte; and finally, they overgrow the benthic communities completely (Ballesteros et al., 2007). The modification of the micro-habitat characteristics of the seagrass beds and the three-dimensional structure of *L. lallemandii* affects the faunal communities associated to the seagrasses.

Rodophyta macroalgae have a rich variety of bioactive metabolites (Blunt et al., 2005). These metabolites posses a wide range of bioactivities such as cytotoxicity (Gross et al., 2006), antimicrobial properties (Vairappan et al., 2004), the capacity to deter herbivores (Vergés et al., 2008) and to inhibit macroalgal surface colonisation

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(antifouling) by macro- and microorganism (Steinberg and de Nys, 2002). *Lophocladia* ssp. are a source of lophocladines, alkaloid molecules with cytotoxic effects (Gross et al., 2006) and known to affect negatively the development of other macroalgaes such as *Caulerpa taxifolia* (Box et al., 2008).

Macroalgae bioactive compounds are considered a possible source of reactive oxygen species (ROS) in macroalgae (Box et al., 2008), seagrasses (Sureda et al., 2008a), fishes (Sureda et al., 2006) and molluscs (Sureda et al., in press). The high production of ROS induces oxidative stress (Tauler et al., 2002; Vina et al., 2000), increasing the markers of lipid peroxidation in target tissues (Alessio, 1993; Alessio and Goldfarb, 1988; Davies et al., 1982; Sureda et al., 2005; Vina et al., 2000). The antioxidant systems protect cells against the deleterious effects by maintaining ROS at relatively low levels and attenuating the damages related to their high reactivity.

Several antioxidant defence mechanisms are present in bivalve molluscs, including low molecular weight compounds (tocopherol, ascorbate, reduced glutathione) and specially adapted enzymes (Winston, 1991). Bivalves express superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which provide cellular defence against endogenous and exogenous ROS. There are differences in the antioxidant defences system between bivalve tissues (Leinö and Lehtonen, 2005; Bocchetti and Regoli, 2006; Box et al., 2007; Soldatov et al., 2007).

The aim of this work was to study whether the presence of *Lophocladia lallemandii* growing on *Pinna nobilis* induces oxidative stress and the antioxidant response in this endangered species and to evaluate also differences in the antioxidant defence system of gills and digestive gland.

2. Materials and methods

2.1. Sampling location

Pinna nobilis specimens were collected in the vicinity of Sa Dragonera Natural Park (SW of Mallorca Island, W Mediterranean; 39°34'48.09'', 2°20'54.57''). Sa Dragonera Island was declared a protected area in 1995 regarding its biodiversity, natural and pristine characteristics. *P. nobilis* is a protected species; therefore, only eight colonised and eight non colonised specimens were collected for the study to diminish damage over *P. nobilis* natural populations. *P. nobilis* was collected at 8–10 m depth by SCUBA diving during summer 2007 according to the maximum *Lophocladia lallemandii* biomasses (Cebrian and Ballesteros, 2007). All individuals of *P. nobilis* had similar dimensions and were collected in the same area and period, the presence or absence of *L. lallemandii* was the difference between specimens. *L. lallemandii* covering *P. nobilis* was collected for biomass determination. With this experimental design, the interference of other factors as geographical differences or water parameter differences (temperature, salinity) are avoided.

Individuals of *Pinna nobilis* without presence of *Lophocladia lallemandii* over shells were considered as control. Colonised and non-colonised *P. nobilis* individuals, with similar size, were collected under license of autonomic institutions (Government of the Balearic Islands).

2.2. Preparation of tissue extracts

Weight and length of *Pinna nobilis* specimens were measured on board. Individuals were also dissected on board in order to remove gills and digestive gland. After dissection, the tissues from each specimen ($n=8$ for each group) were immediately placed on ice and stored frozen at −70 °C. At the laboratory, tissue samples were homogenized in ten volumes (w/v) of 100 mM Tris–HCl buffer (pH 7.5). Each homogenate was sonicated briefly (2–3 s) using ultrasonic processor and centrifuged at 9000 g at 4 °C for 15 min (Manduzio et al., 2004). After centrifugation, supernatants were collected and immediately used for the biochemical analyses. All assays were performed in duplicate.

2.3. Biochemical assays

Malondialdehyde (MDA), as marker of lipid peroxidation (Janero, 1990), was determined by a colorimetric assay kit (Calbiochem, San Diego, CA, USA) following the manufacturer's instructions.

Thioredoxin reductase (TR) activity was measured with an end-point method by thioredoxin coupled insulin reduction assay (Arnér et al., 1999). Absorbance at 412 nm was determined after incubation at 37°C for 20 min. CAT activity (k/mg protein) was measured by the method of Aebi (1984) based on the decomposition of H_2O_2. SOD activity (pmol/min/mg protein) was determined by the degree of inhibition on the reduction of cytochrome c by superoxide anion generated by the xanthine oxidase/hypoxanthine following the method described by McCord and Fridovich (1969). GPX activity (nmol/min/mg protein) was measured using an adaptation of the method of Flohe and Gunzler (1984); this activity was determined with H_2O_2 as substrate and Glutathione reductase (GR) and NADPH as enzyme and non-enzymatic indicators, respectively. Glutathione *S*-transferase (GST) activity was measured by the method of Habig et al. (1974) using reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates.

Enzymatic activities and MDA were determined with a Shimadzu UV-2100 spectrophotometer at 37 °C. MDA concentration and all enzyme activities were measured in duplicate for each sample. Total protein content was determined by a colorimetric method (Biorad Protein Assay) using bovine serum albumin (BSA) as standard to normalize all biochemical results.

2.4. Statistical analysis

Statistical analysis was carried out using a statistical package (STATISTICA® 6.0). The statistical significance between tissues (gills and digestive gland) in control conditions as well as the differences between colonised and non colonised *P. nobilis* was compared by two-way analysis of variance (ANOVA). The possible bivariate correlations (Pearson correlation) between the different parameters (both for colonised and non-colonised *Pinna nobilis*) were also analysed. Results are expressed as mean ± S.E.M. and $p<0.05$ was considered statistically significant.

3. Results

Control specimens had a mean shell height of 34.1 ± 2.3 cm and a mean width of 15.6 ± 0.7 cm whereas the colonised individuals had a

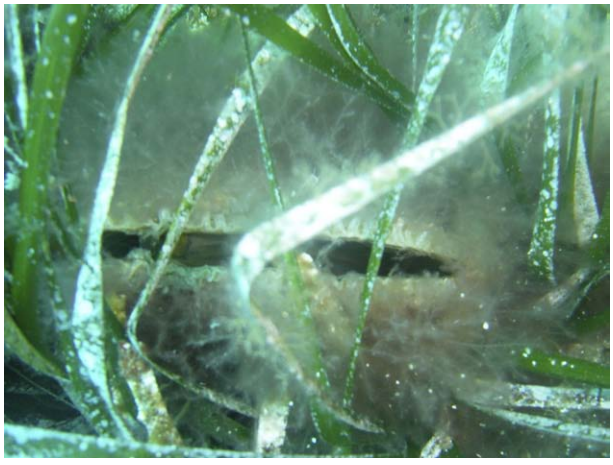

Fig. 1. Frontal upper image of *Pinna nobilis* shell colonised by *Lophocladia lallemandii*, in which it can be observed that the fan mussel is completely colonised and covered by the seaweed.