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Comparative Biochemistry and Physiology, Part C 144 (2007) 348–355
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Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo(*a*)pyrene

R. Ramos, E. García *

Laboratorio de Comunidades Marinas y Ecotoxicología; Departamento Biología de Organismos, Universidad Simón Bolívar, Apdo. 89000, Caracas 1080-A, Venezuela

Received 17 May 2006; received in revised form 6 November 2006; accepted 7 November 2006
Available online 25 November 2006

Abstract

Components of the cytochrome P_{450} monooxygenase system (MFO) and antioxidant enzymes were investigated in the coral *Montastraea faveolata* exposed to the organic contaminant benzo(*a*)pyrene (B(*a*)P). For bioassays the corals were exposed to increasing concentrations of B(*a*)P (0.01 and 0.1 ppm) for 24 and 72 h, with water renewal every 24 h. Enzymatic activity of catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) were measured in host (polyp) and hosted (zooxanthellae) cells. NADPH cytochrome *c* reductase activity and contents of cytochrome P_{450} and P_{420} were only measured in the polyp. Antioxidant enzymes CAT and SOD in polyps and zooxanthellae and GST in polyps increased significantly at the highest concentration and maximum time of exposure. Cytochrome P_{420} was found in all colonies, and the cytochrome P_{450} content was greatest in the colonies from the highest concentrations of contaminant. NADPH cytochrome *c* reductase activity and the concentration of pigments did not vary between treatments. This is the first report of the induction of both detoxifying mechanisms, the MFO system and antioxidant enzymes on acute exposure to an organic contaminant in the reef-constructing coral species *M. faveolata*.

Keywords: Corals; PAHs; Benzo(*a*)pyrene; Antioxidant enzymes; Cytochrome P_{450} system; Biomarkers

1. Introduction

The mixed-function oxygenase (MFO) system, along with its main component the cytochrome P_{450} monooxygenase, is present in all living organisms and plays a fundamental role in the biotransformation of endogenous compounds (i.e., steroids, fatty acids, prostaglandins) and of a variety of exogenous compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochloride pesticides (Den Besten et al., 1990; Solé and Livingstone, 2005). The P_{450} cytochrome catalyzes the first step of the monooxigenation reaction in non-polar organic compounds by transferring electrons from the cytochrome P_{450} NADPH reductase enzyme complex in the microsomal membranes or vesicles (Wright and Welbourn, 2002). The subsequent reactions (corresponding to Phase 2) conjugate the products of the P_{450} system with hydrophilic endogenous molecules. The results of these conjugations are compounds more polar than the parental compounds, and thus, more easily excreted from the organisms (Lee, 1982).

The MFO system has been described in many marine invertebrates (Snyder, 2000) and its induction is currently used as an indicator of the exposure to contaminating substances (Fossi et al., 1998; Cajaraville et al., 2000). In molluscs (Gilewicz et al., 1984; Livingstone, 1988; Porte and Albagaiges, 1993), annelids (Lee, 1998; Galindo, 2004) and crustaceans (James and Boyle, 1998) the MFO system is induced during exposure to xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). PAHs are highly persistent in marine sediments, accumulating in living tissues. MFO metabolism of PAHs leads to intermediate metabolites that strongly react with organic molecules, including proteins and nucleic acids (inducing carcinogenesis), leading to tissue damage and dysfunction (Wright and Welbourn, 2002). Some of these metabolites are reactive oxygen species (ROS) that could in turn induce the antioxidant defense mechanism(s) in these marine organisms (Regoli et al., 2002). The enzymes that constitute the MFO regulate the production of oxyradicals like

* Corresponding author. Tel.: +58 212 9063415; fax: +58 212 9063416.
E-mail address: emgarcia@usb.ve (E. García).

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doi:10.1016/j.cbpc.2006.11.006

the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Winston and Di Giulio, 1991). This enzymatic response represents an adaptive mechanism that is induced by different environmental stressors (*e.g.* organic compounds, heavy metals, temperature, light), and it is less specific than those of the biotransformation enzymes. The study of both the MFO and the biotransformatin enzymes provides a better understanding of the toxic effect of contaminants and the response of the organisms (Livingstone, 1991). The cytochrome P_{450} contents, the activities of biotransformation enzymes (cytochrome *c* NADPH reductase, glutathione S-transferase) and those of the antioxidant enzymes (CAT, SOD and glutathione peroxidase) have all been correlated with increases of PAHs in tissues of various bivalve species, hence they have been regarded as biomarkers of PAHs exposure (Solé et al., 1994; Cheung et al., 2001; De Luca-Abbott et al., 2005).

In cnidarians, the MFO system has been shown to be present under natural conditions in six anemone species (Hefferman et al., 1996; Hefferman and Winston, 1998; Solé and Livingstone, 2005) and in the three scleractinian corals *Favia fragum, Siderastrea siderea* and *Montastraea faveolata* (Gassman and Kennedy, 1992; García et al., 2005). In contrast, Firman (1995) did not detect the MFO system in *M. faveolata* exposed for 90 days to the pesticide chlorane. In order to use the MFO system and the oxidative-stress enzymes as biomarkers of exposure to chemicals in ecosystems such as coral reefs, it becomes necessary to establish a relationship between the presence of contaminants in the surrounding waters and the biochemical responses of the organisms (Cajaraville et al., 1998). However, to date, studies on scleractinian corals have shown that some of their biochemical responses (oxidative-stress enzymes, antioxidant potential, thermal-stress proteins and cellular integrity components) are also influenced by changes in natural conditions such as light and temperature (Brown et al., 2002; Downs et al., 2005; Griffin and Bhagooli, 2004).

Coral reefs are highly productive ecosystems and they furnish economical benefits to nearby human populations (Hoegh-Guldberg, 1999). The continuous increase of polluting substances from man-made sources, the human developments along coastal areas, overfishing, and other global factors have changed water quality, which in turn is causing a generalized deterioration of reef ecosystems (Brown, 1997; Brown et al., 2002; McCulloch et al., 2003). In this study, we evaluated experimentally the induction of cytochrome P_{450}, the enzymatic activities of cytochrome *c* NADPH reductase, glutathione S-transferase, catalase and superoxide dismutase in *M. faveolata* exposed to benzo(*a*)pyrene at sublethal concentrations. Our results showed that induction of the biotransformation and of the antioxidant systems in this coral depended on both the concentration of the contaminant in water and the length of exposure.

2. Materials and methods

2.1. Chemicals

Benzo(*a*)pyrene, henceforth referred to as B(*a*)P, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), cytochrome *c*, glutathione (GSH), 1-chloro,2,4-dinitrobenzene, monobasic potassium phosphate, di-potassium hydrogen phosphate, phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), hypoxanthine, xanthine oxidase and potassium cyanide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethylene diaminetetraacetic acid (EDTA) was obtained from Research Organics, Inc. (Cleveland, USA). Iodoacetamine, pestatin A, leupeptin, aprotinin were obtained from Calbiochem EDM Biosciences, Inc. (Darmstadt, Germany). Sucrose, β-mercaptoethanol, and bovine serum albumin were obtained from Bio-Rad Corp. (San Diego, CA, USA). Hydrogen peroxide and dimethyl sulfoxide (DMSO) were obtained from Riedel de Haën AG (Seelze, Germany).

2.2. Animal collection and experimental conditions

Fragments of *M. faveolata* were collected at Cayo Paicla, Morrocoy National Park, Venezuela (10° 52′ N, 69° 16′ W) in February 2005. The mean surface area of the fragments was 9.93 ± 2.76 cm^2. Fragments were transported to the laboratory in closed 45-L plastic containers with aeration. Before they were placed separately into aerated closed 3.5-L glass aquaria, fragments were cleaned of associated biota such as sponges, polychaetes and algae. Seawater was renewed daily and filtered through fiberglass wool, activated carbon, a phytoplankton net and Whatman No. 1 filter paper. Acclimation period was 3 days and the bioassays were carried out for 24 and 72 h.

Three colonies were used for each dose level exposition. These levels were: acclimation control (C1), 24 h control (C2), 72 h control (C3), 24 h with DMSO (C4), 72 h with DMSO (C5), 24 h and 72 h with 0.01 ppm of B(*a*)P (T1 and T2, respectively), 24 h and 72 h with 0.1 ppm of B(*a*)P (T3 and T4, respectively).

The aquaria were kept aerated during the bioassays and they were covered with translucent plastic. Coral fragments were maintained under a regime of 12 h light: 12 h dark, with an irradiance of 10 μE/m^2 (Li-cor LI-250, underwater sensor UWQ6068). Water temperature (°C), salinity (‰), dissolved oxygen (mg/l), total dissolved solids (g/L) and pH were recorded daily (Hydrolab B, minisonde SURVEYOR 4[a]). During the bioassays the conditions were: 26.07 ± 0.81 °C, 37.48 ± 0.27‰, 5.25 ± 0.18 mgO_2/L, 35.99 ± 0.23 g/L of dissolved solids, and pH 8.21 ± 0.03. All coral fragments were frozen in liquid nitrogen at the end of experimental periods. Afterwards, the samples were stored at −80 °C.

2.3. Tissue preparation

2.3.1. Polyps

Coral tissues were removed from the coral skeleton with pressurized air, and immediately submerged and homogenized in two volumes of homogenization buffer on ice with an Ultra-Turrax blender. The homogenization buffer was 100 mM potassium phosphate at pH 7.6, containing 125 mM sucrose, 1 mM EDTA, 1 mM PMSF, 5 mM β-mercaptoethanol, 0.1 mM aprotinin, 1 μg/mL pepstatin A, and 1 μg/mL leupeptin. Homogenates were centrifuged for 5 min at 2000 ×*g* at 4 °C, to

remove zooxanthellae and traces of skeletal tissue. Zooxanthellae were stored at −80 °C. Coral tissues were homogenized five times ×30 s at 4 °C with a Potter–Elvehjem glass-teflon homogenizer in one volume of cold homogenization buffer. Homogenates were then serially centrifuged for 20 min at 10,000 ×*g*. The pellets were discarded. The supernatant was then centrifuged for 1 h at 100,000 ×*g* to pellet the microsomal fraction. Microsomes were resuspended in 1 mL of homogenization buffer plus 20% glycerol (v/v). The supernatant and microsomes were stored at −80 °C.

2.3.2. Zooxanthellae

A volume of 1 mL of zooxanthellae was centrifuged for 6 min at 2000 ×*g;* the supernatant was discarded and the pellet was resuspended in 1 mL of cold 20 mM HEPES (*N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid]) and 0.15 M KCl at pH 7.4, containing 1 mM EDTA, 1 mM PMSF, 5 mM β-mercaptoethanol, 10 mM iodoacetamine, and 2 mM benzamidine. Zooxanthellae were homogenized twice ×30 s and sonicated for 20 min in cold solution. Homogenates were serially centrifuged for 20 min at 10,000 ×*g*. The pellets were discarded. The supernatant was then centrifuged for 30 min at 20,000 ×*g*. Protein concentrations of tissues and zooxanthellae were determined by the micro-Bradford method (Bradford, 1976).

2.4. Enzyme assays

2.4.1. Cytochromes P_{450} and P_{420}

These were measured according to Omura and Sato (1964). The carbon monoxide (CO) differential spectrum was recorded by adding sodium dithionite (DTN) and performing a background correction prior to the addition of CO. P_{450} and P_{420} contents were determined in a 1 cm cuvette containing approximately 300–500 μg of microsomal protein solubilized with 100 mM potassium phosphate at pH 7.6, containing 125 mM sucrose, 1 mM EDTA, 1 mM PMSF, and 5 mM β-mercaptoethanol. The spectra were scanned (350–500 nm) at room temperature (25–27 °C) in a Perkin Elmer Lambda 35 dual-beam spectrophotometer. The P_{420} and P_{450} concentrations were calculated using their respective extinction coefficients ($\varepsilon_{420-490}=110\ mM^{-1}\ cm^{-1}$ and $\varepsilon_{420-490}=91\ mM^{-1}\ cm^{-1}$).

2.4.2. NADPH cytochrome c reductase

The activity of NADPH cytochrome *c* reductase was measured in the microsomal fraction, following the method described by Hefferman and Winston (1998). Final assay concentrations in a volume of 1 mL were: 0.05 mM cytochrome *c*, 1 mM potassium cyanide, approximately 150–250 μg microsomal protein and 0.05 mg/mL NADPH in 100 mM potassium phosphate at pH 7.7.

2.4.3. Glutathione S-transferase (GST)

The activity of glutathione S-transferase was determined following the method described by Habig et al. (1974), using the 100,000 ×*g* supernatant. The standard assay for glutathione S-transferase activity used 1-chloro-2,4-dinitrobenzene. Final assay concentrations in a volume of 1 mL were: 1 mM 1-chloro-2,4-dinitrobenzene, 1 mM glutathione (GSH), 100 mM potassium phosphate buffer at pH 6.5.

2.4.4. Catalase (CAT)

The activity of catalase was determined following the method described by Aebi (1974) and using the 100,000 ×*g* supernatant. Final assay concentrations in a volume of 3 mL were: 150 mM H_2O_2 and 80 mM potassium phosphate buffer at pH 7.0.

2.4.5. Superoxide dismutase (SOD)

The activity of superoxide dismutase was determined following the method described by McCord and Fridovich (1969) and using the 100,000 ×*g* supernatant. The activity of this enzyme is given in SOD units (1 unit = 50% of inhibition of the xanthine oxidase reaction). Final assay concentrations in a final volume of 1 mL were: 50 mM potassium phosphate buffer at pH 7.8, 0.1 mM EDTA, 90 μM cytochrome *c*, 0.1 mM hypoxanthine, and 1.86 mμ/mL xanthine oxidase. Biochemical measurements of activity were carried out on the Perkin Elmer spectrophotometer. For each enzyme the samples were measured three times, except NADPH cytochrome *c* reductase samples, which were measured twice.

2.5. Zooxanthellae pigments and density

The concentration of chlorophyll *a*, *c*1 + *c*2 and pheopigment was determined following the method described by Arar (1997) and the content of carotenoids was estimated as in Parsons and Strickland (1963). Aliquots of 0.5 mL of zooxanthellae were centrifuged for 6 min at 2000 ×*g*. The pellets were resuspended in a glass tube of 5 mL with 2 mL 90% acetone at 4 °C in the dark for 12 h. Extracts were centrifuged for 6 min at 2000 ×*g*. The supernatant was read immediately at 750, 665, 664, 663, 647 and 630 nm, in a glass cuvette in a spectrophotometer.

The total density of zooxanthellae was determined on seven subsamples of 10 μL of the total volume of zooxanthellae using a Neubauer haemocytometer. The density was expressed in number of cells × cm^2 of area of each colony. The colony area was estimated using the aluminum foil method (Marsh, 1970).

2.6. Statistical analyses

The parametric one-way ANOVA and Duncan's multiple-range tests (STATISTICA 6.0), were used to compare differences between the bioassays treatment levels and a $p<0.05$ was considered significant.

3. Results

3.1. Cytochrome P_{450} and P_{420}

Fifty-six percent of the total colonies showed a 450 nm peak, with a mean of 0.36 ± 0.54 nmol/mg microsomal protein, and 85% of the colonies had a 420 nm peak, with a mean of 1.07 ± 1.15 nmol/ mg microsomal protein (Table 1). All levels of treatment, except the control with solvent DMSO at 72 h (C5),

Table 1
Contents of microsomal protein, cytochromes P_{420} and P_{450}, and enzymatic activity of NADPH cytochrome *c* reductase, in the microsomal fractions of *Montastraea faveolata* for each treatment

Treatments	Protein microsomal mg/mL	P_{420}	P_{450}	NADPH cytochrome *c* reductase nmol/min/mg. microsomal protein
		nmol /mg microsomal protein		
C1	0.57±0.21	0.41±0.71	0.04±0.08	114.14±145.54
C2	0.47±0.33	0.17±0.15	ND	212.83±198.46
C3	0.40±0.09	1.76±0.93	ND	212.36±244.44
C4	0.49±0.22	0.60±0.58	0.35±0.60	8.71±12.74
C5	0.87±0.79	ND	ND	0.89 ±1.39
T1	0.72±0.29	1.12±1.23	ND	27.34±27.20
T2	0.46±0.15	2.23±2.00	0.07±0.11	25.00±27.07
T3	0.49±0.23	0.85±1.31	0.73±0.70	1.53±1.35
T4	0.65±0.44	1.04±1.10	0.61±0.77	125.38±189.86

C1: control of acclimation, C2: control at 24 h, C3: control at 72 h, C4: control with DMSO 24 h, C5: control with DMSO 72 h, T1: 0.01 ppm at B(*a*)P 24 h; T2: 0.01 ppm at B(*a*)P 72 h; T3: 0.1 ppm at B(*a*) P 24 h; T4: 0.1 ppm at B(*a*)P 72 h. All values are mean±S.D. ($n=3$). ND: not detectable.

showed the cytochrome P_{420}. However, cytochrome P_{450} content was greater in colonies from the highest concentrations of contaminant (T3 and T4).

3.2. Enzyme activity in the polyp tissue

NADPH cytochrome *c* reductase activity showed differences among treatments (ANOVA, $p=0.02$). The treatments C1, C2, C3 and T4 showed higher values than any other treatment (Duncan's test, $p<0.01$, Table 1). In treatment C5 (control with solvent DMSO at 72 h) only one out of three fragments showed enzymatic activity. These results were consistent with the above results, where cytochrome P_{450} and P_{420} were not detected in the C5 treatment.

Glutathione S-transferase activity also differed among treatments (ANOVA, $p<0.001$), being highest in the T4 assay (Duncan's test, $p<0.01$, Fig. 1). This latter treatment had the highest concentration of B(*a*)P (0.1 ppm) and the lengthiest time of exposure (72 h).

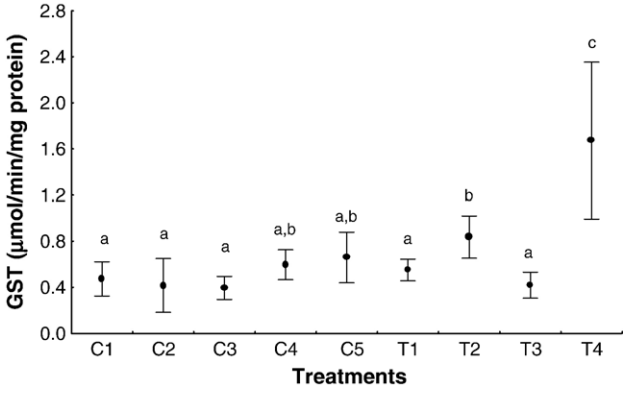

Fig. 1. Mean enzymatic activity (±S.D., $n=3$) of glutathione S-transferase in the zooxanthellae-free tissue of *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time. Abbreviations as in Table 1. Different letters indicate significant differences (Duncan's test, $p<0.01$).

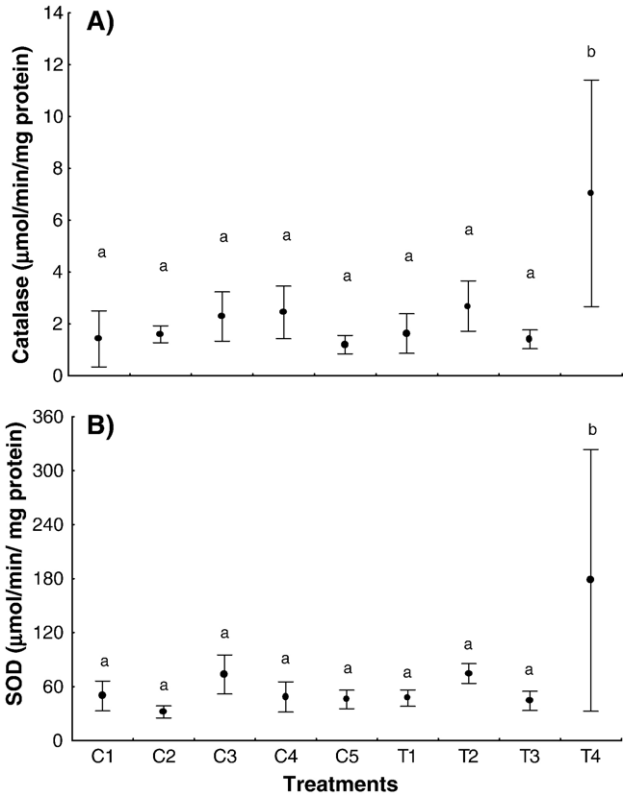

Fig. 2. Mean (±S.D., $n=3$) activities of antioxidant enzymes in the zooxanthellae-free tissue of *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time. A) Catalase, B) Superoxide dismutase. Further details as in Fig. 1.

The activity of the antioxidant enzymes catalase and superoxide dismutase differed among treatments (ANOVA, $p<0.001$), and was highest in the test T4 (Duncan's test, $p<0.01$, Fig. 2A and B).

3.3. Enzyme activity in the zooxanthellae

In contrast with the activity of glutathione S-transferase in the polyp tissue, this enzyme showed no significant differences

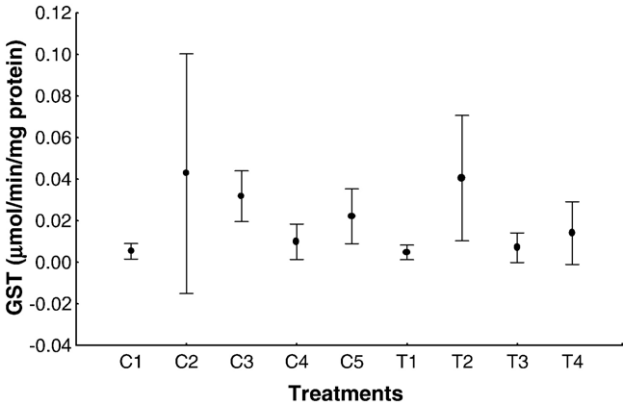

Fig. 3. Mean enzymatic activity (±S.D., $n=3$) of glutathione S-transferase in the zooxanthellae of *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time. No differences were detected among treatments.

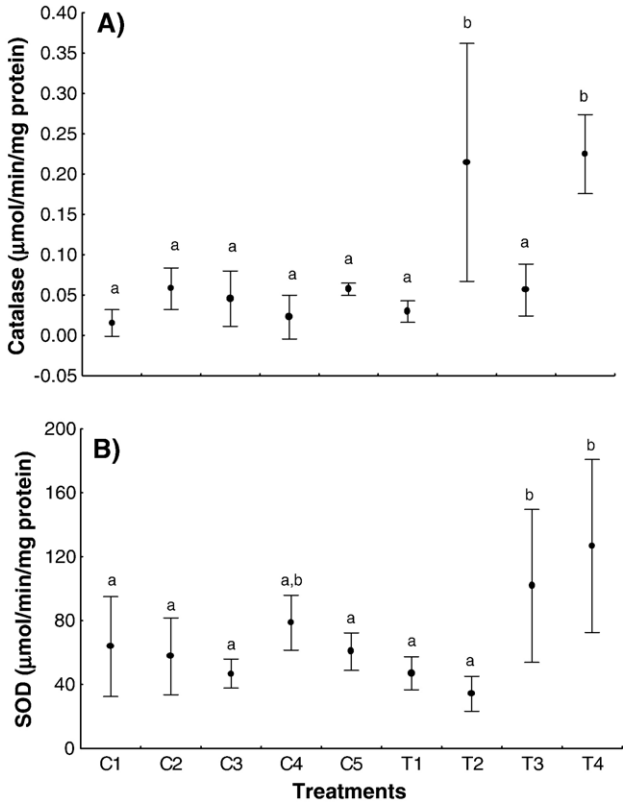

Fig. 4. Mean (±S.D., $n=3$) activities of antioxidant enzymes in the zooxanthellae of *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time. A) Catalase, B) Superoxide dismutase. Further details as in Fig. 1.

in its activity among treatments in these hosted cells. Furthermore, the mean activity was lower than that of the polyp tissue (0.02±0.03 µmol/min/mg protein) (Fig. 3). At the same time, the activity of the antioxidant catalase and superoxide dismutase enzymes showed the same trend than in the polyp tissue, with the highest activity at the highest B(*a*)P concentration and longest exposure time (T4) (Duncan test, $p<0.05$, Fig. 4A and B). The mean catalase activity was lower than in the polyp

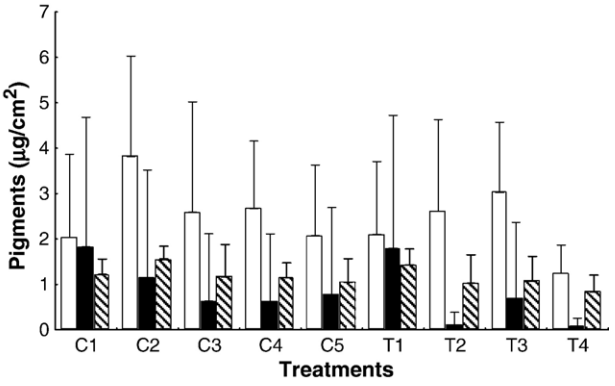

Fig. 5. Zooxanthellae pigment content (±S.D., $n=3$), based on coral surface area of *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time. White bars: chl *a*, black bars: pheopigments and striped bars: chl *c*. No differences were detected among treatments.

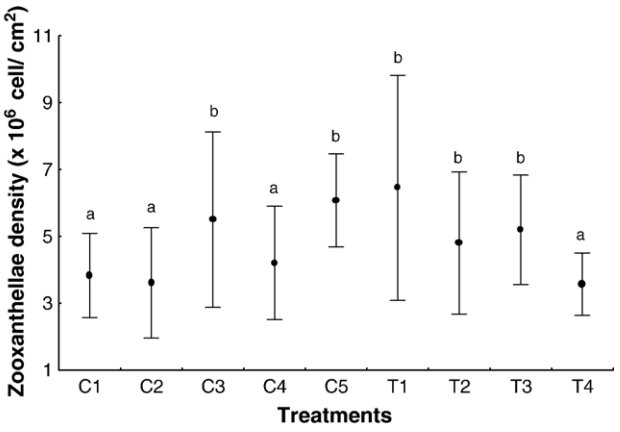

Fig. 6. Zooxanthellae density (±S.D., $n=9$) per surface area of the coral *Montastraea faveolata* in relation to B(*a*)P concentration and exposure time to. Different letters indicate significant differences (Duncan's test, $p<0.01$). Abbreviations as in Table 1.

tissue (0.09±0.10 µmol/min/mg protein), whereas the mean superoxide dismutase activity was similar to that of polyp tissue (68.55±37.07 µmol/min/mg protein).

3.4. Zooxanthellae pigment and density

No significant differences between treatments were detected for the concentration of pigments in the zooxanthellae. The mean concentrations of chlorophyll *a* and *c* were 2.46±1.77 µg/cm^2 and 1.17±0.48 µg/cm^2 respectively. Pheopigment concentration was lowest for the longest exposure times (T2 and T4), with a mean of 0.85±1.87 µg/cm^2 (Fig. 5). The content of carotenoids was similar among treatments, with a mean of 5.19±1.84 µg/cm^2.

The mean zooxanthellae density was of 4.85±2.30×10^6 cell/cm^2. The highest density was recorded at the lowest concentration and the shortest exposure (T1), and the lowest density at the highest concentration of B(*a*)P and the longest exposure (T4) (Duncan's test, $p<0.01$, Fig. 6). Despite differences in zooxanthellae density, colonies showed no signs of bleaching.

4. Discussion

The findings of this study clearly show the induction of biotransformation and antioxidant enzymes in *M. faveolata* exposed to the organic compound benzo(*a*)pyrene. Our results revealed that the mean concentration for cytochromes P_{450} and P_{420} in *M. faveolata* exposed to B(*a*)P were two or three orders of magnitude greater than those reported for other cnidarian species (Gassman and Kennedy, 1992; Hefferman and Winston, 1998; Solé and Livingstone, 2005), while being similar to those previously found in *S. siderea* and *M. faveolata* under natural conditions (García et al., 2005). Compared with other marine invertebrates, the content of cytochrome P_{450} in *M. faveolata* falls within the range reported for molluscs, echinoderms and annelids (Den Besten et al., 1990; Livingstone, 1991; Solé and Livingstone, 2005). Contrarily to cytochrome P_{450}, P_{420} was present in all treatments. This ubiquity of cytochrome P_{420} agrees with previous results in *M. faveolata* under natural

conditions (García et al., 2005), as well as with those from other invertebrates, including sponges, molluscs, annelids, crustaceans and echinoderms, in which the peak at 418 nm has been shown together with that at 450 nm (Solé and Livingstone, 2005). Despite this, in a bivalve mollusc population impacted by high hydrocarbon levels the 418 nm peak was conspicuous while no activity was detected at 450 nm (Moore et al., 1987). The polychaete *Armandia maculata* showed only the 420 nm activity peak under natural conditions, whereas *Sabella melanostigma* showed an activity peak at 420 nm and a larger one at 450 nm on exposure to 0.1 ppm of B(*a*)P for seven days (Galindo, 2004).

Differing from the behaviour of cytochrome P_{450}, the enzymatic activity of cytochrome *c* NADPH reductase was greater in the controls C1 to C3 than in the exposure treatments; this difference reflects alterations of the enzyme levels. Although a similar behavior between cytochrome P_{450} and the NADPH reductase is to be expected, this does not always hold true. As found in this study, in the bivalve mollusc *Mytilus* the latter diminished in specimens that contained the highest organic compound and cytochrome P_{450} levels (Livingstone, 1988). Furthermore, while cytochrome *c* NADPH reductase and PAHs content varied in direct proportion to each other in clams and mussels, this was not the case in oysters (Solé et al., 1994); likewise, while in the gasteropod *Buccinum undatum* the cytochrome *c* NADPH reductase was not linked to elevated organic compound contents in the tissues, the NADH reductase activity increased (Santos et al., 2002).

The greater activity of GST in the polyp tissue of *M. faveolata* exposed to the highest concentration of B(*a*)P for the longest period activated the expression of the MFO system. During the Phase 1 metabolism, in which the epoxidation of B(*a*)P by cytochrome P_{450} takes place, the intermediate 7,8 diol 9, 10 epoxide (which is highly reactive with organic molecules) becomes conjugated with glutathione by way of the GST enzyme, rendering it less reactive and more hydrophilic (Cheung et al., 2001; Wright and Welbourn, 2002). Even though GST has been proposed as one of the main enzymes for the study of biotransformation of xenobiotic substances in marine organisms (Lee, 1998; Vrolijk and Targett, 1992; Cheung et al., 2001), a clear direct relationship between this enzyme and PAHs has only been demonstrated for compounds with five or six rings, including B(*a*)P (Gowland et al., 2002).

Organic pollutants such as PAHs induce the generation of intracellular free radicals by means of oxidative mechanisms or systems such as the cytochrome P_{450} (Livingstone, 1988; Regoli et al., 2002). The induction of enzymatic activity of antioxidant complexes like catalase and superoxide dismutase in *M. faveolata* polyps clearly indicates a response of antioxidant defenses in coral colonies exposed to 0.1 ppm B(*a*)P for 72 h. In the zooxanthellae the induction of CAT and SOD also occurred, but GST and CAT activities were smaller than in the polyps. This agrees with reports of CAT enzyme in *Palythoa caribaeorum* (Lesser et al., 1990) and *Favia favus* (Levy et al., 2006). In our study GST activity in zooxanthellae did not show any relationship with the exposure to B(*a*)P. This might indicate that B(*a*)P is being metabolized to Phase I, in the host cells in the first place, rather than in the hosted cells. However, these results differ from findings concerning GST and CAT in *Montastraea annularis* (in a bathymetric gradient) in which zooxanthellae showed higher enzymatic activities than polyp tissues (Downs et al., 2005).

The antioxidant enzymes CAT and SOD also increased in the zooxanthellae in the treatments with the longest exposure and the highest B(*a*)P concentration, thus supporting that this hydrocarbon leads to an oxidative state in both compartments of *M. faveolata* coral*:* polyps and zooxanthellae. Downs et al. (2002), had already proposed that free radicals can cross the membranes of zooxanthellae towards their host cells. Hence, we propose that free radicals would be produced in the cytosol of polyp cells due to the biotransformation of B(*a*)P and cross towards the zooxanthellae, thereby activating their antioxidant defenses. Differing from the polyp cells, zooxanthellae are photosynthetically active and are highly prone to generate ROS (Lesser, 1996). Therefore, the antioxidant defense in these organisms is one of the main mechanisms of regulation and/or reaction that are induced by stress conditions. This induction has been reported in the corals *M. annularis* and *Goniastrea aspera* during temperature and irradiation stress (Brown et al., 2002; Downs et al., 2002).

The zooxanthellae density found in the fragments of *M. faveolata* in our study, 4.85×10^6 cells cm^{-2}, agrees well with values reported for colonies of this species and of *M. annularis* at depths between 3 and 6 m (Szmant and Gassman, 1990; Warner et al., 1999). Likewise, the chlorophylls *a* and *c* contents also fell within the normal range found in other corals, whereas the carotenoid levels in our experimental conditions were above those under natural conditions (Kleppel et al., 1989). Although the pigments of the zooxanthellae did not vary significantly on exposure to B(*a*)P at its highest concentration and the longest exposure, the levels of chlorophylls *a* and *c*, pheopigments and carotenoids per coral surface area tended to diminish. This might be caused by zooxanthellae damage, or their expulsion or death (Gleason and Wellington, 1993; Lesser, 1996).

Slow rates of accumulation and clearance of hydrocarbons such as lindane, phenantrene and phtalate esters in the scleractinian coral *Diploria strigosa* (Knap et al., 1982; Solbakken et al., 1985) supported the idea that this species was capable to metabolize such compounds. However, our results provide for the first time evidence that the presence of an organic contaminant can induce short-term activation of detoxifying mechanisms in the coral species *M. faveolata*. This response involves the MFO system in the host cells, i.e. polyps, and a mechanism against the oxyradical production in both the host and the hosted cells (polyps and zooxanthellae, respectively). The induction of the MFO system and the antioxidant defenses could be used as an early warning or as a biomarker of contamination by PAHs in the main reef-constructing coral species.

Acknowledgements

We would like to thank C. Bosque, D. Debrot, S. Rodriguez and C. Bastidas for critical review of this work. O. Farina for

assistance in laboratory and field work. This research was supported by FONACIT, under project S1-2001000368.

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